

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



IFW#
DAC

U.S. PATENT AND TRADEMARK OFFICE

In the application of:)	Examiner: TBA
LEDBETTER et al.)	Group Art Unit: TBA
Serial No.: 10/627,556)	
Filed: July 26, 2003)	
For: BINDING CONSTRUCTS)	
AND METHODS OF USE)	
THEREOF)	

TRANSMITTAL LETTER

Mail Stop Petitions
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Enclosed for filing for the above-referenced application please find:

1. Petition for Suspension of Rules under 37 C.F.R. § 1.183;
2. Declarations of:
 - A. Barbara J. Kielt;
 - B. James W. Collett, Ph.D. (includes Exhibit A, copy of U.S. application entitled "Binding Constructs and Methods of Use Thereof," now assigned U.S. Serial No. 10/627,556);
 - C. Michelle Hon;

CERTIFICATE OF MAILING
(37 C.F.R. Section 1.8A)

I hereby certify that this paper (along with anything referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as First Class Mail in an envelope addressed to the Mail Stop Petitions, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

June 25, 2004
Date of Deposit

Barbara J. Kielt
Name of Person Mailing Paper
Barbara J. Kielt

Signature of Person Mailing Paper

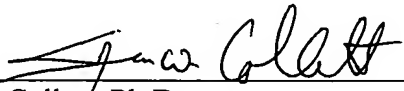
D. Mildred Pampena

E. Dale Stuckey, Jr.; and

F. John Norton.

The Commissioner is hereby authorized to charge Deposit Account No. 02-4553 in the amount of \$130.00. If this fee is incorrect, the Commissioner is hereby authorized to debit or credit Deposit Account No. 02-4553 accordingly.

Respectfully submitted,



James W. Collett, Ph.D.
Reg. No. 46,636

BUCHANAN INGERSOLL P.C.
One Oxford Centre
301 Grant Street
Pittsburgh, PA 15219-1410
(619) 578-5000
Direct: (619) 578-5054
Fax: (619) 578-5940
Email: collettjw@bipc.com

Patents
49076.000004.CIP2

A Petition fee as set forth in 37 CFR 1.17(h) in the amount of \$130.00 is believed due. The Commissioner is authorized to debit the amount of \$ 130.00 from Deposit Account No. 02-4553 in payment of the Petition fee as set forth in 37 C.F.R § 1.17(h). In the event any additional amounts are due in connection with the filing of this Petition, the Commissioner is further authorized to debit the additional amounts.

Included with this Petition are the following Declarations:

1. **Declaration of Barbara Kielt**, administrative assistant/paralegal with Buchanan Ingersoll LLP (Kielt Decl.);
2. **Declaration of James W. Collett, Ph.D.**, Associate Attorney with Buchanan Ingersoll LLP (Collett Decl.);
3. **Declaration of Michelle Hon**, a Summer Associate with Buchanan Ingersoll LLP (Hon Decl.);
4. **Declaration of Mildred Pampena**, Manager of Networks and Communications with Buchanan Ingersoll PC (Pampena Decl.);
5. **Declaration of United States Post Office employee Dale Stuckey, Jr.** (Stuckey Decl.); and,
6. **Declaration of United States Post Office employee John Norton** (Norton Decl.).

This request for a correction of filing date has arisen from extraordinary circumstances that require intervention by the United States Patent and Trademark Office to correct an injustice that will result if the filing date is not corrected.

Background

The application that is the subject of this Petition represents foundational technology of Trubion Pharmaceuticals, Inc., a start-up biotechnology company located in Seattle, Washington. The inventions described and claimed in the application relate to immunology and, more specifically, to immunotherapeutics for use in the treatment of a number

of serious disorders relating to the unwanted proliferation of cells, including cancers and autoimmune disorders, such as rheumatoid arthritis. Trubion began operations and received Series A funding in November 2002. It is now seeking Series B funding, and currently employs 32 people, all of whom are located in Seattle.

The subject application, titled "Binding Constructs and Methods of Use Thereof," is 526 pages long. It relates to "Small Modular Immunotherapeutics" comprising a target binding domain polypeptide, a hinge region polypeptide and an effector function polypeptide comprising, for example, immunoglobulin CH2 and CH3 polypeptide sequences. The application describes over 100 different specific immunotherapeutic protein constructs, and contains 71 Figures in 75 sheets of drawings, 160 Examples, and 697 nucleotide and protein sequences (Collett Decl., Exhibit A). The application is a Continuation-in-Part of U.S. Serial No. 10/053,530, filed January 17, 2002 (entitled "Binding Domain-Immunoglobulin Fusion Proteins"), and also claims priority to U.S. Provisional Application No. 60/385,691 filed on June 3, 2002 (entitled "Recombinant Signaling Receptors for Tumor Gene Therapy").

Trubion Pharmaceuticals has obtained significant results from use of constructs of the invention in preclinical animal studies. For example, company scientists have shown in preclinical studies that TRU015, a construct that is covered by this patent application, has an anti-disease function that is at least equivalent to, if not better than, that of Rituxan®, an anti-cancer therapeutic currently marketed by Genentech that generates sales of over \$1.3 billion annually. Trubion plans to file an Investigative New Drug application (an "IND") this summer in order to pursue human clinical trials prior to marketing this new drug to patients.

**Request For Waiver Of Rule 10
Due To Extraordinary Circumstances
And In Order To Meet The Requirements Of Justice**

Rule 183 (37 C.F.R. § 1.183) provides in pertinent part:

In an extraordinary situation, when justice requires, any requirement of the regulations in this part which is not a requirement of the statutes may be suspended or waived by the Director or the Director's designee, *sua sponte*, or on petition of the interested party, subject to such other requirements as may be imposed.

Applicants respectfully request that the Commissioner suspend Rule 1.10 regarding Express Mail and award a filing date of July 25, 2003, the date the application was presented to the Post Office for filing but not accepted. Due to a combination of unforeseeable factors, this is a situation of extraordinary circumstances. Justice requires this earlier date because, without the correction of the filing date to July 25, 2003, PCT Application No. PCT/US02/01487, published July 25, 2002, Publication No. WO 02/056910 would become available for citation as a reference under 35 U.S.C. § 102(b), it having been published one year and one day before the official filing date of July 26, 2003 that was given to the present application.

Rule 10, entitled, "Filing of Correspondence by 'Express Mail,'" provides that, subject to certain exceptions not applicable here:

Any correspondence received by the U.S. Patent and Trademark Office (USPTO) that was delivered by the "Express Mail Post Office to Addressee" service of the United States Postal Service (USPS) will be considered filed with the USPTO on the date of deposit with the USPS, . . .

37 C.F. R. § 1.10(a)(1). The rule further provides in subsection (a)(2) that "[t]he date of deposit with USPS is shown by the 'date in' on the 'Express Mail' label or other official USPS notation." 37 C.F.R. § 1.10(a)(2). Additionally, subsection (b) states that "[c]orrespondence should be deposited directly with an employee of the USPS to ensure that the person depositing

the correspondence receives a legible copy of the ‘Express Mail’ mailing label with the ‘date-in’ clearly marked.” 37 C.F.R. § 1.10(b).

In this instance, several unforeseeable factors combined to block Applicants from obtaining a “date-in” of July 25, 2003, the date their application was presented to the Post Office for delivery to the PTO by Express Mail. Instead, Applicants were given a “date-in” of July 26, 2003. The first factor is the refusal of the Post Office to accept Applicants’ Express Mail packages, one of which contained the subject application. Although presented in person on July 25, 2004, the Post Office would not accept the packaged patent applications, stating that they were late because the hours of the main United States Post Office in San Diego (located near the airport on Midway Drive) had changed so that it was open only until 11:00 p.m., rather than midnight, its previous closing time.

As set forth in her Declaration accompanying this Petition, Barbara Kielt is a paralegal with Applicants’ law firm, Buchanan Ingersoll LLP. Ms. Kielt has been a legal administrative assistant since 1973, over 30 years, and has over eight years of direct patent prosecution experience. (Kielt Decl. ¶ 2). As noted in her Declaration, part of Ms. Kielt’s duties over her years of patent experience was to assist in the preparation and mailing of patent applications (Kielt Decl. ¶ 3). During this time she has known and on a number of occasions taken advantage of the fact that the San Diego’s main Post Office was open to accept Express Mail packages until midnight and had employees present to provide a “date-in” stamp on deposit any time before midnight (Kielt Decl. ¶¶ 4, 5 and 11). She reasonably relied on the fact that the main branch of the San Diego Post Office had been open until midnight for acceptance of Express Mail packages for years and that it would be open until midnight on July 25, 2003 (Kielt Decl. ¶¶ 1-5).

On the night of July 25, 2003, Ms. Kielt arrived at the Midway Post Office at approximately 11:40 p.m., well before the midnight cut-off for Express Mail (Kielt Decl. ¶ 11; Stuckey Decl. ¶¶ 3, 4; Norton Decl. P¶¶ 4,5). Ms. Kielt's arrival is confirmed and verified by the Declaration of Post Office employee Dale Stuckey, Jr. However, she was informed by Mr. Stuckey, who was behind the counter at the time, that the Post Office had changed its hours and that it only accepted packages presented by 11:00 p.m. (Kielt Decl. ¶ 12 ; Stuckey Decl. ¶ 4; Norton Decl. ¶ 5). Even though a Post Office employee, Mr. Stuckey, was working at that time, he would not accept the package (Kielt Decl. ¶ 12; Stuckey Decl. ¶ 4; Norton Decl. ¶ 5).

Another event on the night of July 25, 2003 was the unexpected shut down of the server that supports all of the computers and printers in the San Diego office of Buchanan Ingersoll LLP at approximately 10:00 p.m. (Collett Decl. ¶ 8; Hon Decl. ¶ 3; Kielt Decl. ¶ 8). The server shut down in the middle of making final typographical corrections and printing the U.S. application ultimately assigned Serial No. 10/627,556, which delayed the completion and printing of the application by at least 40 minutes (Collett Decl. ¶ 8; Kielt Decl. ¶ 8). It was unforeseeable to the personnel in the San Diego office of Buchanan Ingersoll that the computer server would shut down while completing and printing the subject U.S. application. Typically, if the Systems Department of Buchanan Ingersoll would shut down the computer system, it would send out notices in advance to allow people to plan for those occasions (Hon Decl. ¶ 5). This time, no notification was received until a few minutes before the server shut down (Collett Decl. ¶ 10). The computers and printers simply stopped working without advance warning (Hon Decl. ¶ 5; Collett Decl. ¶ 10) and were unavailable for over 30 minutes (Collett Decl. ¶ 8; Hon Decl. ¶ 3; Kielt Decl. ¶ 8). It was later learned that it was the practice of the Pittsburgh-based Systems Department of Buchanan Ingersoll to shut down the server nightly at 1:00 a.m. Eastern time

(Hon Decl. ¶ 6; Pampena Decl. ¶ 2) – which corresponds to 10:00 p.m. San Diego time – for maintenance. However, given that the intellectual property personnel in the San Diego office of Buchanan Ingersoll had only just recently joined the firm (Collett Decl. ¶ 11), and that this was the first time that any one in that group had been using the computers at 10:00 p.m. since joining the firm (Collett Decl. ¶ 12), they had no way of knowing about the daily practice of the Pittsburgh Systems Department and were, therefore, unable to plan ahead for the unavailability of their computers and printers. The San Diego office is the only office of Buchanan Ingersoll that is not in the Eastern time zone. Thus, the shut-down of the computers and printers on July 25 was not something that could have been prevented.

These circumstances combined to constitute a “Perfect Storm” of extraordinary and unforeseeable events. They were not due to simple inadvertence, and but for the change in hours at the Midway Post Office and the refusal of the Post Office to accept Applicants’ Express Mail package, despite the presence of an employee who could have, the package containing the patent application at issue would have been date stamped on July 25, 2003, the date it was presented to the Post Office pursuant to PTO rules.

Although “extraordinary circumstances” is not defined in the rules, the MPEP states that inadvertent failure to comply with requirements is not considered extraordinary. MPEP 500-61. Here, there was no inadvertent failure to comply with requirements.

Extraordinary circumstances have been found in a variety of cases. *See, e.g., In re Application of Hardee*, 223 U.S.P.Q. 1122 (Comr. 1984)(granting suspension of 37 C.F.R. § 1.48 under Rule 183 to allow removal of a co-inventor without co-inventor's consent upon showing proof of sole inventorship); *Helgott v. Director of the U.S. P.T.O.*, 209 F.3d 1328 (Fed.

Cir. 2000) (stating that a Rule 183 request should be granted to remedy mistakes made after PTO accidentally changed the agent's file reference number).

On the other hand, extraordinary circumstances are typically not found in cases where there is attorney neglect, ignorance of the law or ignorance of due dates. See, e.g., *In re: Reexamination of U.S. Patent No. 3,963,842*, 227 U.S.P.Q. 255 (Comr. 1985) (unawareness of patent laws and due dates is not an extraordinary circumstance, citing *Nugent v. Yellow Cab*, 295 F.2d 794 (7th Cir. 1961)); *Gustafson v. Strange*, 227 U.S.P.Q. 174 (Comr. 1985) (ignorance of 37 C.F.R. 1.8 is not extraordinary). Here, there was no attorney neglect, no ignorance of the law, and no ignorance of due dates. Indeed, as set forth in the Declaration of James W. Collett, Ph.D., Applicants' attorneys were working diligently on the preparation of this complicated patent application for several months, spending nearly 60 hours on its preparation in April and May 2003, over 80 hours working on drafts in June 2003, and over 300 hours finalizing various drafts in July 2003, including 59 hours during the first week of July, 75 hours during the second week in July, 108 hours during the third week in July, and 98 hours during the week of July 20, the week it was due to be filed.

The instant situation is different from that in *Nitto Chemical Industry v. Commr*, 39 U.S.P.Q.2D 1778 (D. D.C. 1994), where, on review of the Commissioner's denial of Nitto's petition to waive Rule 10, the court determined that there were no extraordinary circumstances justifying the waiver. In that case, an attorney who had finished two patent applications reportedly gave them to a secretary who prepared two Express Mail packages. The secretary then gave them to an assistant manager for the firm's mail contractor (Betancourt) who signed the Express Mail certifications attesting that the packages were to be deposited with the USPS for delivery to the PTO via Express Mail service. Betancourt then returned the certifications to

the attorney and gave the packages to another employee of the mail contractor (Perez) to deliver to the Post Office. The Declaration of Perez stated that he thought the mail he was given contained only first class mail but did not know whether it contained any Express Mail packages. All of the first class mail delivered by Perez to the Post Office was ultimately delivered to the appropriate destinations, but the PTO never received any of eight separate Express Mail packages, including the two at issue in the case.

The Commissioner denied applicant's request to waive the Rule 10 filing requirements because "there was no persuasive evidence that the original application papers were, in fact, deposited with the USPS on February 29, 1991" and because the "mishandling of the Express Mail packages amounted to an 'oversight that could have been prevented by the exercise of ordinary care or diligence'." *Nitto*, 39 U.S.P.Q.2d at 1778. On appeal, the district court affirmed denial of the petition. First, the district court found unpersuasive Nitto's argument that it had provided documentary evidence equivalent to a certificate of mailing because the Rule 10 requirement that a certificate of mailing be signed and dated by the person mailing the application was not met. In fact, the person who signed the certificate (Betancourt) did not mail them, but instead gave the packages to someone else (Perez) to mail.

The district court also ruled that it was not "unreasonable for the Commissioner to conclude that Nitto's evidence was not as probative of the mailing date of the packages as an Express Mail receipt." *Nitto*, 39 U.S.P.Q.2d at 1778. Nitto's evidence included an affidavit of Betancourt, who stated that he had put the Express Mail packages in the evening mailbag that he said he handed to Perez, and an affidavit of Perez stating that he deposited the entire contents of the mailbag at the Post Office.

Finally, the district court held that Nitto did not submit any evidence showing that the inventions claimed in the patent applications at issue would be denied patentability and, consequently, the Commissioner had no basis on which to determine whether "justice required" him to award the earlier filing date. 39 U.S.P.Q.2d at 177.

The present situation is distinguishable in every respect from that of *Nitto*.

1. First, in *Nitto* there was no evidence that the packages were deposited in Express Mail (instead, Perez's Declaration said that he had no specific recollection of taking Express Mail packages to the Post Office on the date in question) and the packages never arrived at the PTO. Here, the packages were deposited in Express Mail and the packages did arrive at the PTO. Applicants have provided Declarations from two Post Office employees and Ms. Kielt demonstrating that on July 25, 2003, Ms. Kielt presented two Express Mail packages addressed to the PTO at the Post Office – one containing the subject patent application – and attempted to deposit them for Express Mail delivery (Kielt Decl. ¶¶ 11-13; Stuckey Decl. ¶¶ 3 and 4; Norton Decl. ¶¶ 5 and 6). Additionally, the Declarations conclusively establish that the same packages with the same contents did arrive at the PTO, as further evidenced by the fact that a serial number has been assigned for the two applications contained in the two packages;
2. Second, in *Nitto*, the court emphasized that one of the reasons that Express Mail receipts are probative is that "a disinterested USPS employee" signs them. *Nitto*, 39 U.S.P.Q.2d at 1780. Here, applicants have provided the signed Declarations of disinterested Post Office employees in support of their Petition;
3. Third, in *Nitto*, the person who signed the certificate of mailing was not the person who allegedly delivered the packages to the Post Office. In the present case, the person who signed the certificate of mailing, Ms. Kielt, did deliver the package to the Post Office (Kielt Decl. ¶¶ 11-13; Stuckey Decl. ¶¶ 3 and 4; Norton Decl. ¶¶ 5 and 6); and,
4. Fourth, unlike *Nitto*, justice requires the earlier filing date because the patentability of the inventions could be adversely affected if this Petition is not granted.

In summary, the circumstances in the present case are a "Perfect Storm" of the unexpected convergence of extraordinary and unforeseeable events noted above and were not due to simple inadvertence. The change in hours at the Midway Post Office, so that it did not accept Express Mail packages after 11:00 p.m., the fact that the server shut down in the middle of printing the U.S. application, and the fact that the San Diego office of Buchanan Ingersoll had

recently added personnel who were unaware that the server routinely shut down at 10:00 p.m. each night, all combined to prevent Ms. Kielt from obtaining a date-in stamp of July 25, 2003, even though the patent application was presented to the U.S. Post Office on that day for filing pursuant to PTO rules.

Justice requires this earlier date because, without the correction of the filing date to July 25, 2003, PCT Application No. PCT/US02/01487, published July 25, 2002 (Publication No. WO 02/056910) would become available for use as a reference under 35 U.S.C. § 102(b), it having been published one year and one day before the filing date given to the present application (currently listed as July 26, 2003, but sought to be corrected to July 25, 2003 by this Petition).

The present application is a continuation-in-part of U.S. Serial No. 10/053,530, filed January 17, 2002. The above-noted published PCT Application (PCT/US02/01487) – which becomes available as a 102(b) reference unless the filing date is corrected – corresponds to the U.S. '530 application and has the same specification. One of the advantages of filing a continuation-in-part application rather than a separate application is that the parent application may not itself be used as prior art against the CIP application. Thus, if the filing date is not corrected to the date the application was presented to the Post Office, as requested by Applicants, the present application is effectively turned into an independent application due to the publication of the PCT application corresponding to its parent. The advantage of filing the present application as a CIP application would be lost and the parent application could be used as a 102(b) reference against the present application.

Conclusion

Based upon the foregoing information and Declarations, Applicants submit that the failure to obtain a July 25, 2003 filing date for the above-referenced patent application was an extraordinary situation that requires the suspension of rule 37 C.F.R. § 1.10 by the Commissioner in the interest of justice. Applicants respectfully request that the filing date of the above-identified application be corrected to July 25, 2003.

Dated: June 25, 2004

Respectfully submitted,

By: James W. Collett
James W. Collett, Esq., Ph.D
Reg. No. 46,646

DECLARATION OF BARBARA J. KIELT

I, Barbara J. Kielt, declare as follows:

1. I am employed as a paralegal in the Intellectual Property Group of the San Diego office of Buchanan Ingersoll LLP, located at 401 West A Street, Suite 1900, San Diego, California. On July 25, 2003, I was employed by Buchanan Ingersoll LLP as an administrative assistant in the Intellectual Property Group of the San Diego Office of Buchanan Ingersoll LLP.

2. Prior to working at Buchanan Ingersoll, I held positions as an intellectual property administrative assistant since 1996, with Brobeck, Phleger & Harrison LLP, Lyon & Lyon LLP, InterVu, Inc., Akamai Technologies, Inc. and Kintera, Inc. I have been a legal administrative assistant since 1973.

3. One of my responsibilities as an intellectual property administrative assistant or paralegal is to prepare and mail patent applications, and I always mail these patent applications by Express Mail through the United States Postal Service.

4. In my employment as an intellectual property administrative assistant over the last several years, I have personally deposited patent applications by Express Mail, and had U.S. Post Office employees present to provide a "date-in" stamp, at the U.S. Post Office, located at 2585 Midway Drive, San Diego between 11:00 p.m. and 12:00 midnight.

5. During these past 8 years as an intellectual property administrative assistant, I have never known the Post Office to change its hours and relied upon the fact that the 2585 Midway Drive Post Office was open until 12:00 midnight for acceptance of Express Mail packages.

6. On Friday, July 25, 2003, I assisted in the preparation of two patent applications: (1) a U.S. Application entitled, "Binding Constructs and Methods of Use Thereof," now assigned U.S. Serial No. 10/627,556; and (2) a PCT application entitled, "Binding Domain-Immunoglobulin Fusion Proteins," now assigned PCT Serial No. PCT/US02/2491, which were to be filed with United States Patent and Trademark Office by Express Mail on that date.

7. The PCT application was ready to be filed by early afternoon on July 25, 2003, and was placed in a sealed package with an Express Mail label filled out at that time, but was held until the U.S. application was ready to be filed so both applications could be taken to the Midway Post office at the same time and mailed by Express Mail.

8. At 10:00 p.m. on July 25, 2003, while we were trying to complete and print the final version of the U.S. patent application, the server supporting all of the computers and printers in the San Diego office of Buchanan Ingersoll disconnected, and we lost all access to the document and were unable to continue. It was not until after sometime after 10:30 p.m. that I was able to access the server, and further time was required to reboot, log on and open the document, leading to the loss of at least 40 minutes due to the server shut-down.

9. All work on the U.S. application, which was 526 pages long and had an additional 75 sheets of drawings, was completed and the patent application was separately packaged and addressed to the United States Patent and Trademark Office to the attention of the appropriate Mail Stop and I immediately left the office at approximately 11:25 p.m..

10. I drove to the U.S. Post Office, located at 2585 Midway Drive, San Diego, CA, in order to deposit these two applications by Express Mail with an employee of the U.S. Post Office before midnight on July 25, 2003.

11. I entered the Post Office at approximately 11:40 p.m. A U.S. postal clerk named Dale Stuckey whom I recognized from several other times I was at this Post Office to make late evening Express Mail filings, was standing at the window where mail is accepted by the Post Office. A bar had been pulled down at this window and it appeared to be closed.

12. Mr. Stuckey informed me that the Midway Post Office had changed its closing hours to 11:00 p.m. and refused to accept my Express Mail packages. When I asked him when the change had occurred, he said that it had been at least several months.

13. I was unable to deposit my two packages containing the patent applications by Express Mail because of Midway Post Office's change of closing time and, therefore, did not obtain a U.S. Patent & Trademark Office filing date of July 25, 2003.


14. After the Post Office's refusal to accept the two Express Mail packages on Friday, July 25, 2003, I attempted unsuccessfully to reach Dr. Collett to obtain instructions.

15. On Saturday morning, July 26, 2003, I contacted Dr. Collett by telephone and explained what had happened at the Post Office. Dr. Collett instructed me to deposit the packages containing the two patent applications by Express Mail with the U.S. Postal Service on Saturday, July 26, 2003.

16. The two patent applications and the entire contents of the two packages deposited with the U.S. Postal Service on Saturday, July 26, 2003 are the same and identical to those prepared and packaged Friday, July 25, 2003.

I declare under penalty of perjury that the foregoing is true and correct.

Dated: June 25, 2004.


Barbara J. Kielt

DECLARATION OF JAMES W. COLLETT, PH.D.

I, James W. Collett, Ph.D., declare as follows:

1. I am employed as an associate in the Intellectual Property Group of the law firm of Buchanan Ingersoll LLP, located at 401 West A Street, Suite 1900, San Diego, California.

2. I have been employed in this position since April of 2003.

3. Prior to this position, I have held positions as an intellectual property attorney for the preceding five years, including positions with Amylin Pharmaceuticals, Inc. and Sheldon & Mak LLP.

4. My responsibilities as an associate in the Intellectual Property Group of Buchanan Ingersoll LLP include the preparation of patent applications and the supervision of Barbara J. Kielt, then an administrative assistant in the Intellectual Property Group of Buchanan Ingersoll LLP.

5. On Friday, July 25, 2003, I assisted in the completion of two patent applications: (1) a U.S. Application entitled, "Binding Constructs and Methods of Use Thereof," now assigned U.S. Serial No. 10/627,556; and (2) a PCT application entitled, "Binding Domain-Immunoglobulin Fusion Proteins," now assigned PCT Serial No. PCT/US02/24918. I supervised Ms. Kielt in preparing these two patent applications to be filed with United States Patent and Trademark Office by Express Mail on that date.

6. The completion and preparation for filing on July 25, 2003, was the culmination of several months of work on the U.S. application. Applicants' attorneys spent nearly 60 hours on its preparation in April and May 2003, over 80 hours working on drafts in June 2003, and over 300 hours finalizing various drafts in July 2003, including 59 hours during the first week of July, 75 hours during the second week in July, 108 hours during the third week in July, and 98 hours during the week of July 20, the week it was due to be filed.

7. The PCT application was ready to be filed by early afternoon on July 25, 2003, but was held until the U.S. application was ready to be filed so both applications could be mailed by Express Mail at the Midway Post office at the same time.

8. At 10:00 p.m. on July 25, 2003, while we were trying to complete and print the final version of the U.S. patent application, the server supporting all of the

computers and printers in the San Diego office of Buchanan Ingersoll LLP disconnected, and we lost all access to the document. It was not until sometime after 10:30 p.m. that we were able to access the server. Further time was required to reboot, log on and open the document, leading to the loss of at least 40 minutes due to the server shut-down. A copy of the U.S. patent application, which consists of 526 pages and 75 sheets of drawings, is attached hereto as Exhibit A.

9. The server is located in the main office of Buchanan Ingersoll PC, which is in Pittsburgh, Pennsylvania, and we had no access to the server or control over it.

10. Prior to July 25, 2003, I had received several notifications from the Systems Department in the Pittsburgh office of Buchanan Ingersoll PC that the server would be shut down for maintenance at various times, typically over a weekend. I received no such notification that this would happen on Friday, July 25, 2003, until a few minutes before the server shut down.

11. Before April, 2003, the San Diego office of Buchanan Ingersoll LLP did not have any intellectual property staff. In April, 2003, Bradford J. Duft joined as a shareholder, and Ms. Kielt as an administrative assistant, in the Intellectual Property Group in the San Diego office. As noted above, I joined the San Diego office of Buchanan Ingersoll LLP as an Associate in the Intellectual Property Group in April 2003.

12. The first time that I or anyone else in the Intellectual Property Group in the San Diego office of Buchanan Ingersoll LLP had tried to use the computers or printers between 10:00 and 10:30 p.m. was July 25, 2003. Consequently, before that date, no one in the Intellectual Property Group in the San Diego office was aware of, what I later learned from Michelle Hon, a Summer Associate with Buchanan Ingersoll LLP, were nightly shut-downs of the server controlling all of the computers and printers in the San Diego office.

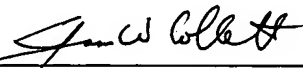
13. All work on the U.S. application was completed in the Buchanan Ingersoll LLP San Diego office and the application was ready to file not later than about 11:25 p.m. Each patent application and its corresponding transmittal papers had been placed in separate packages by Ms. Kielt and each package was addressed to the United States Patent and Trademark Office to the appropriate Mail Stop. Ms. Kielt left at that

time to drive to the Midway Drive Post Office to deposit the two packages by Express Mail.

14. Ms. Kielt contacted me the next morning, Saturday, July 26, 2003, and explained what had happened at the Post Office the evening before, namely, that a Post Office employee was at the Post Office when Ms. Kielt arrived with the packages but that he had refused to accept them, stating that the branch had changed its Express Mail deposit cut-off time from midnight to 11:00 p.m. I instructed Ms. Kielt to deposit the two packages by Express Mail with the U.S. Postal Service on Saturday, July 26, 2003.

I declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge.

Dated: June 25, 2004.



James W. Collett, Ph.D.

EXHIBIT A

Copy of U.S. Application entitled, "Binding Constructs and Methods of Use Thereof,"
now assigned U.S. Serial No. 10/627,556.

BINDING CONSTRUCTS AND METHODS FOR USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a Continuation-in-Part of U.S. Serial No. 10/053,530, filed
5 January 17, 2002, which is incorporated herein by reference in its entirety and which claims
the benefit of priority of U.S. Provisional Application Serial No. 60/367,358 (formerly U.S.
Serial No. 09/765,208, filed January 17, 2001), also incorporated herein by reference in its
entirety and for which a Petition to Convert a Non-Provisional Application to a Provisional
Application was filed on January 16, 2002, and granted on June 6, 2002. This application
10 also claims the benefit of priority of U.S. Provisional Application No. 60/385,691 filed on
June 3, 2002, and entitled "Recombinant Signaling Receptors for Tumor Gene Therapy,"
which is also incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

15 The present invention relates generally to compounds having various utilities
including uses for research, diagnostics, and therapy, for example, immunotherapy.
Compounds of the invention include immunologically active proteins and protein
conjugates. Such proteins include recombinant or engineered binding proteins such as, for
example, binding domain-immunoglobulin fusion proteins, which may include single chain
20 Fv-immunoglobulin fusion proteins and compounds containing single chain Fv-
immunoglobulins. The present invention also relates to compositions and methods for
treating conditions, diseases and disorders that would improved, eased, or lessened from the
administration of, for example, polypeptide and/or nucleic acid constructs of the invention,
including, for example, malignant conditions and B cell disorders, including diseases
25 characterized by autoantibody production and/or inflammation.

BACKGROUND OF THE INVENTION

The immune system is one of the most complex of the body's many intricate
systems. A vast and complicated arrangement made up of many different types of cells and
30 involving many different kinds of molecules, the human immune system allows the body to

respond to foreign invaders such as bacteria, viruses, and other infectious agents, as well as foreign material such as pollen. In general, the human immune system is divided into two main parts, antibody-mediated immunity (also called “humoral” or “circulating” immunity) and cell-mediated immunity, both of which are managed by lymphocytes. Lymphocytes are one of the five kinds of white blood cells (leukocytes) circulating in the blood. There are several kinds of lymphocytes, each with different functions to perform. The most common types of lymphocytes are B lymphocytes (B cells), which are responsible for making antibodies, and T lymphocytes (T cells). Cells of the immune system not only include T cells and B cells, but also Natural Killer Cells, granulocytes (or polymorphonuclear (PMN) leukocytes), macrophages, and dendritic cells. The humoral system is managed by B cells with help from T cells and deals with infectious agents in the blood and tissues of the body. The cell-mediated system is managed by T cells and deals with cells of the body that have been infected.

An antigen is a substance, usually macromolecular, that stimulates or induces an immune response. Because of its complex macromolecular structure, a single microorganism consists of multiple antigens (*e.g.*, surface structures such as cell wall components, fimbriae, flagella, *etc.*, or extracellular proteins, such as toxins or enzymes produced by the microorganism). The coat proteins and some of the envelope proteins of animal viruses are also usually antigenic. A host is generally able to respond specifically to antigens that come into contact with components of its immune system. Both the antibody-mediated immunity and cell-mediated immunity systems involve complex interrelationships that allow them to mount immune reactions to almost any antigen. In other words, the immune system is able to recognize foreign substances (antigens) that stimulate the system to produce antibody-mediated immunity, cell-mediated immunity, or both.

The immune system complex is constituted by a variety of different cell types and organs disseminated throughout the body. These include the primary lymphoid organs, *i.e.*, the bone marrow and the thymus. All the cells of the immune system are initially derived from the bone marrow in a process called hematopoiesis. During hematopoiesis bone marrow-derived stem cells differentiate into either mature cells of the immune system (“B” cells) or into precursors of cells that migrate out of the bone marrow to mature in the

thymus (“T” cells). In addition to red blood cells, platelets, and B cells, the bone marrow also produces Natural Killer cells, granulocytes, and immature thymocytes. The function of the thymus is to produce mature T cells. Immature thymocytes, also known as prothymocytes, leave the bone marrow and migrate into the thymus where they mature and are then released into the bloodstream.

The immune system complex also includes secondary lymphoid organs, *e.g.*, the spleen, the lymph nodes, *etc.*, as well as a circulatory system that is separate from blood vessels. The spleen, made up of B cells, T cells, macrophages, dendritic cells, Natural Killer cells, and red blood cells, is an immunologic filter of the blood. In addition to capturing antigens from the blood that passes through the spleen, migratory macrophages and dendritic cells bring antigens to the spleen via the bloodstream. An immune response is initiated in the spleen when macrophages or dendritic cells present the antigen to the appropriate B or T cells, and B cells become activated and produce large amounts of antibody. Lymphatic vessels and lymph nodes are the parts of a special circulatory system that carries lymph, a transparent fluid containing white blood cells, chiefly lymphocytes. Lymph bathes the tissues of the body, and is then collected in lymphatic vessels. Lymph nodes dot the network of lymphatic vessels and function as an immunologic filter for lymph when afferent lymph ducts bring lymph-containing antigens into the node. Composed mostly of T cells, B cells, dendritic cells, and macrophages, the lymph nodes drain fluid from most tissues. Antigens are filtered out of the lymph in the lymph node before the lymph is returned to the circulation. Macrophages and dendritic cells that capture antigens also present these foreign materials to T and B cells in the lymph nodes, resulting in the stimulation of B cells to develop there into antibody-secreting plasma cells. Antibodies leave the lymph node by the efferent ducts that empty into the blood stream. Lymphocytes can also leave the node by the efferent duct and travel to other sites in the lymphatic system or enter into the blood circulation. A single lymphocyte completes a circuit through the circulating blood and lymphatic systems once every 24 hours.

Tonsils, adenoids, Peyer’s patches, and the appendix are also lymphoid tissues. Peyer’s patches (masses of lymphocytes) are similar to the tonsils and are found throughout the body, especially in the mucous linings of the digestive and respiratory tracts.

It is the function of the phagocytic cells found in Peyer's patches and other lymphatic aggregate follicles to defend the body against, for example, inadequately digested food particles crossing the gut wall and entering the blood, and to attack unwanted foreign invaders while they are still in the bowel.

5 The major function of B cells is the production of antibodies in response to foreign proteins of bacteria, viruses, and tumor cells. T cells are usually divided into two major groups, namely, the cytotoxic T lymphocytes ("Tc" cells or CTLs) and the helper T cells ("Th" cells or T helper cells). Th cells, also referred to as CD4+ T cells, function to augment or potentiate immune responses by the secretion of specialized factors that activate
10 other white blood cells to fight off infection. They enhance the production of antibodies by B cells. Tc cells, also called CD8+ T cells, can directly kill certain tumor cells, viral-infected cells, and sometimes parasites. Tc cells are also important in down-regulation of immune responses. Both types of T cells often depend on the secondary lymphoid organs (the lymph nodes and spleen) as sites where activation occurs, but they are also found in
15 other tissues of the body, including the liver, lung, blood, and intestinal and reproductive tracts.

Natural Killer cells, often referred to as NK cells, represent another type of lymphocyte and are similar to the Tc cell subset. They function as effector cells that directly kill certain tumors such as melanomas and lymphomas, and viral-infected cells. They are
20 called "natural" killers because, unlike cytotoxic T cells, they do not need to recognize a specific antigen before carrying out their function. While NK cells, unlike the Tc cells, kill their targets without prior activation in the lymphoid organs, NK cells activated by Th cell secretions will kill tumor or viral-infected targets more effectively. NK cells target tumor cells and protect against a wide variety of infectious microbes. In several immunodeficiency
25 diseases, including AIDS, Natural Killer cell function is abnormal. Natural Killer cells may also contribute to immunoregulation by secreting high levels of influential lymphokines. Some NK cells have surface receptors (FcγRIII, also called CD16) for the Fc portion of the IgG antibody. They bind to target cells through receptors for the Fc portion of an antibody that has reacted with antigen on a target cell. This type of cell-mediated immunity is called
30 antibody-dependent cell-mediated cytotoxicity (ADCC). NK cells may also have receptors

for the C3 component of complement, another immune defense system, and therefore recognize cells that are coated with C3 as targets. ADCC is thought to be an important defense against a variety of parasitic infections caused, for example, by protozoa and helminths.

5 Although small lymphocytes look identical, they can be distinguished by molecules carried on their cell surface. Not only do such markers distinguish between B cells and T cells, they distinguish among various subsets of cells that behave differently. Every mature T cell, for instance, carries a marker known as T3 (or CD3). In addition, most helper T cells carry a T4 (CD4) marker, a molecule that recognizes Class II major
10 histocompatibility complex ("MHC") antigens. A molecule known as T8 (CD8), which recognizes Class I MHC antigens, is found on many suppressor/cytotoxic T cells.

 Another group of white blood cells collectively referred to as granulocytes, or polymorphonuclear leukocytes (PMNs), is composed of three cell types. These cells, neutrophils, eosinophils, and basophils are important in the removal of bacteria and parasites
15 from the body. Neutrophils migrate through capillary walls and into infected tissue where they kill invaders (*e.g.*, bacteria) and then engulf the remnants by phagocytosis. Eosinophils are cytotoxic, releasing the contents of their granules on an invader. Basophils leave the blood and accumulate at the site of an infection or other inflammation and discharge the contents of their granules, releasing a variety of mediators such as histamine, serotonin,
20 prostaglandins and leukotrienes that, for example, increase blood flow to the area. Mediators released by basophils also play an important part in some allergic responses such as hay fever and anaphylactic responses to insect stings.

 Monocytes are large phagocytic white blood cells released from the bone marrow into the blood circulation. When a monocyte enters tissue, it develops into a
25 macrophage. Macrophages are also large, phagocytic cells that engulf foreign material (antigens) that enter the body, as well as dead and dying cells of the body. Macrophages are important in the regulation of immune responses, and are often referred to as scavengers, or antigen-presenting cells (APCs) because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T cells and B cells. This
30 is one of the important first steps in the initiation of an immune response. Stimulated

macrophages exhibit increased levels of phagocytosis and also secrete Interleukin-1 (IL-1), a product that helps to activate B cells and T cells.

Dendritic cells also originate in the bone marrow and function as APCs. They are usually found in the structural compartment of lymphoid organs such as the thymus, lymph nodes and spleen, but are also found in the bloodstream and other tissues. It is believed that dendritic cells capture antigen or bring it to the lymphoid organs where an immune response is initiated.

Important features of the immunological system relevant to host defense and/or immunity to pathogenic microorganisms include specificity, memory, and tolerance. It is understood, for example, that an antibody or reactive T cell will react specifically with the antigen that induced its formation; it will not react with other antigens. Generally, this specificity is of the same order as that of enzyme-substrate specificity or receptor-ligand specificity, although cross-reactivity is possible. The specificity of the immune response is explained by clonal selection. During the primary immune response, a specific antigen selects a pre-existing clone of specific lymphocytes and stimulates its activation, proliferation and differentiation. It is also understood that once the immune system has responded to produce a specific type of antibody or reactive T cell, it is capable of producing more of the antibody or activated T cell more rapidly and in larger amounts; this is called the secondary (or memory) response. It is also recognized that an animal generally does not undergo an immunological response to its own (potentially-antigenic) components. The animal is said to be tolerant, or unable to react to its own potentially antigenic components. This ensures that under normal conditions, an immune response to “self” antigens (called an autoimmune response) does not occur. Tolerance is brought about in a number of ways, but in essence the immune system is able to distinguish “self” components from “non-self” (foreign) antigens; it will respond to “non-self” but not to “self”. Sometimes in an animal, tolerance can be “broken”, which may result in an autoimmune disease.

The biological activities of the antibody-mediated and cell-mediated immune responses are different and vary from one type of infection to another. There are several classes or types of antibodies (and subclasses of various types) involved in antibody-mediated immunity. All of the classes of antibodies that are produced in response to a

specific antigen react stereochemically with that antigen and not with other (different) antigens. The host has the genetic capacity to produce specific antibodies to thousands of different antigens, but does not do so until there is an appropriate (specific) antigenic stimulus. Due to clonal selection, the host produces only the homologous antibodies that will react with that antigen which, as noted above, are found in blood (plasma), lymph, and many extravascular tissues. Once the antibody-mediated immunity response occurs following interaction of B lymphocytes with antigen and their differentiation into antibody-secreting plasma cells, the secreted antibody binds to the antigen which, in turn, results in its neutralization or elimination from the body.

Cell-mediated immunity, on the other hand, is mediated by specific subpopulations of T-lymphocytes called effector T cells that exist in precursor form as “resting T cells” (pT cells). These cells bear receptors for specific antigens and recognize these antigens on the surfaces of other cells. Stimulation with that antigen results in T cell activation. T cells enlarge, enter into a mitotic cycle, reproduce and develop into effector T cells whose activities are responsible for this type of immunity. They also develop into clones of identical reactive T cells called memory T cells. As noted above, most of the T cells in the body belong to one of two subsets and are distinguished by the presence on their surface of one or the other of two glycoproteins designated CD4 and CD8. Which of these molecules is present determines the types of cells to which the T cell can bind. T cells bearing CD8 ($CD8^+$ T cells) always recognize antigen in association with Class I MHC proteins and typically function as cytotoxic T cells. Almost all the cells of the body express Class I MHC molecules. T cells bearing CD4 ($CD4^+$ T cells) always recognize antigens in association with Class II MHC proteins on the surfaces of other cells. Only specialized antigen-presenting cells express Class II MHC molecules, including dendritic cells, phagocytic cells such as macrophages, and B cells. $CD4^+$ T lymphocytes generally function as T helper cells.

T helper cells, which include Th1 cells and Th2 cells, respond to antigen with the production of lymphokines. Th1 and Th2 cells can be distinguished based on their lymphokine profiles. Like all T cells, Th cells arise in the thymus. When they are presented with an antigen by antigen-presenting dendritic cells they begin to proliferate and become

activated. There are two kinds of dendritic cell, DC1 cells (descended from monocytes) and DC2 cells (which appear to be derived from lymphocytes).

Th1 cells (inflammatory Th1 cells involved in the elimination of pathogens residing intracellularly in vesicular compartments) are produced when DC1-type dendritic cells present antigen to the T cell receptor for antigen (TCR) and secrete Interleukin 12 (IL-12). This paracrine stimulation activates Th1 cells to secrete their own lymphokines, in particular, Tumor-Necrosis Factor-beta (TNF- β) (also known as lymphotoxin) and Interferon-gamma (IFN- γ). These lymphokines stimulate macrophages to kill bacteria they have engulfed by phagocytosis and they recruit other leukocytes to the site producing inflammation. Th1 cells are essential for cell-mediated immunity and for controlling intracellular pathogens such as, for example, *Listeria* and *Mycobacterium tuberculosis*.

Th2 cells ("true" helper Th2 cells, which are required for antibody production by B cells) are produced when DC2-type dendritic cells present antigen to the T cell receptor for antigen and, presumably, one or more paracrine stimulants. The major lymphokines secreted by Th2 cells are Interleukin 4 (IL-4), which stimulates class-switching in B cells and promotes their synthesis of IgE antibodies, acts as a positive-feedback device promoting more pre-Th cells to enter the Th2 pathway, and blocks expression of the IL-12 receptor thereby inhibiting pre-Th cells in the thymus from entering the Th1 pathway. IL-4 also causes B cells to proliferate and differentiate into antibody-secreting plasma cells and memory B cells. IL-4 activates only B cells in the vicinity which themselves have bound the antigen, and not others, so as to sustain the specificity of the immune response. Th2 cells also produce Interleukin 5 (IL-5, which attracts and activates eosinophils), Interleukin 10 (IL-10, which inhibits IL-12 production by DCs and prevents maturation of pre-Th cells to Th1 cells), and Interleukin 13 (IL-13, which also promotes the synthesis of IgE antibodies).

Activation of the Th2 cell also causes it to begin to produce Interleukin 2 (IL-2), and to express a membrane receptor for IL-2. The secreted IL-2 autostimulates proliferation of Th2 cells. For example, IL-2 binds to IL-2 receptors on other T cells (which have bound the antigen) and stimulates their proliferation. In addition to IL-2, stimulated Th2 cells also produce IFN- γ and Interleukin 6 (IL-6), which mediate various aspects of the immune response. IFN- γ activates Natural Killer cells to their full cytolytic potential, and

is an activator of macrophages and thus increases their antitumor activities. If the macrophages are infected by intracellular parasites, it activates macrophages which in turn destroy the parasites. IFN- γ also reinforces the antitumor activities of the cytotoxic lymphocytes, increases the nonspecific activities of NK-cells, and is one of the factors that controls the differentiation of B cells and increases the secretion of immunoglobins. IL-6 stimulates several types of leukocytes, as well as the production of Acute Phase Proteins in the liver. It is particularly important in inducing B cells to differentiate into antibody forming (plasma) cells. Thus, Th2 cells provide help for B cells and are essential for antibody-mediated immunity.

Cytotoxic T lymphocytes are able to kill cells that show a new or foreign antigen on their surface (for example, virus-infected cells, or tumor cells, or transplanted tissue cells). The CD8⁺ CTLs also come in two subsets: Tc1 that, like Th1 cells, secrete IFN- γ , and Tc2 that, like Th2 cells, secrete IL-4.

The cell-mediated immunity response also plays a role in destruction of tumor cells and in rejection of tissue transplants in animals. A major problem in tissue transplantation is rejection, which is often based on cell-mediated immunity response to “foreign” cells (because they are not a perfect antigenic match). Because tumor cells contain specific antigens not seen on normal cells they also may be recognized as foreign and destroyed by the forces of cell-mediated immunity. If tumor cells develop on a regular basis in animals, it may be cell-mediated immunity that eliminates them or holds them in check. The increase in the incidence of many types of cancer (tumors) in humans with advancement of age may be correlated with a decline in the peak efficiency of the immune system that occurs about 25 years of age.

A summary of the types of cells involved in the expression of cell-mediated immunity follows. Tc lymphocytes kill cells bearing foreign antigen on surface in association with Class I MHC and can kill cells that are harboring intracellular parasites (either bacteria or viruses) as long as the infected cell is displaying a microbial antigen on its surface. Tc cells kill tumor cells and account for rejection of transplanted cells. Tc cells recognize antigen-Class I MHC complexes on target cells, contact them, and release the contents of granules directly into the target cell membrane which lyses the cell. Th

lymphocytes produce lymphokines that are “helper” factors for development of B cells into antibody-secreting plasma cells. They also produce certain lymphokines that stimulate the differentiation of effector T lymphocytes and the activity of macrophages. Th1 cells recognize antigen on macrophages in association with Class II MHC and become activated (by IL-1) to produce lymphokines including IFN- γ that activates macrophages and NK cells. These cells mediate various aspects of the cell-mediated immunity response including delayed-type hypersensitivity reactions. Th2 cells recognize antigen in association with Class II MHC on an APC and then produce interleukins and other substances that stimulate specific B cell and T cell proliferation and activity. Macrophages are important as antigen-presenting cells that initiate T cell interactions, development, and proliferation. Macrophages are also involved in expression of cell-mediated immunity because they become activated by IFN- γ produced in a cell-mediated immunity response. Activated macrophages have increased phagocytic potential and release soluble substances that cause inflammation and destroy many bacteria and other cells. Natural Killer cells are cytotoxic cells that lyse cells bearing new antigen regardless of their MHC type and even lyse some cells that bear no MHC proteins. NK cells are defined by their ability to kill cells displaying a foreign antigen (*e.g.*, tumor cells) regardless of MHC type and regardless of previous sensitization (exposure) to the antigen. NK cells can be activated by IL-2 and IFN- γ , and lyse cells in the same manner as cytotoxic T lymphocytes. Some NK cells have receptors for the Fc domain of the IgG antibody and are thus able to bind to the Fc portion of IgG on the surface of a target cell and release cytolytic components that kill the target cell via antibody-dependent cell-mediated cytotoxicity.

Extracellular factors that affect cell proliferation and differentiation have been defined as cytokines. These include the lymphokines, which are proteins produced by T-lymphocytes that have effects on the differentiation, proliferation, and activity of various cells involved in the expression of cell-mediated immunity. In general, lymphokines function by (1) focusing circulating leukocytes and lymphocytes into the site of immunological encounter; (2) stimulating the development and proliferation of B cells and T cells; (3) stimulating and preparing macrophages for their phagocytic tasks; (4) stimulating Natural Killer cells; and (5) providing antiviral cover and activity. A summary of various

important lymphokines follows. Initially referred to as lymphocyte activation factor, IL-1 is mainly a product of macrophages, and has a variety of effects on various types of cells. It acts as a growth regulator of T cells and B cells, and it induces other cells such as hepatocytes to produce proteins relevant to host defense. IL-1 forms a chemotactic gradient for neutrophils and serves as an endogenous pyrogen which produces fever. Thus, IL-1 plays an important role in both the immune responses and in the inflammatory response. IL-2 stimulates the proliferation of T cells and activates NK cells. IL-3 regulates the proliferation of stem cells and the differentiation of mast cells. IL-4 causes B cell proliferation and enhanced antibody synthesis. IL-6 (also referred to as Interferon-beta2, hybridoma growth factor, B-cell differentiation factor, and hepatocyte stimulatory factor) has effects on B cell differentiation and on antibody production and on T cell activation, growth, and differentiation, and probably has a major role in the mediation of the inflammatory and immune responses initiated by infection or injury. IL-8 is a chemotactic attractant for neutrophils. IL-13 shares many of the properties of IL-4, and is a potent regulator of inflammatory and immune responses. IFN- γ is produced by T cells and may be considered a lymphokine. It is sometimes called "immune interferon" (Interferon-alpha being referred to as "leukocyte interferon" and Interferon-beta being referred to as "fibroblast interferon"). IFN- γ has several antiviral effects including inhibition of viral protein synthesis in infected cells. It also activates macrophages and NK cells, and stimulates IL-1, IL-2, and antibody production. Lymphotoxins include the Tumor Necrosis Factors. TNF-beta is produced by T cells, while TNF-alpha is produced by T cells as well as other types of cells. TNFs function to kill cells, including tumor cells (at a distance). There are several Colony Stimulating Factors (CSFs), including granulocyte macrophage colony stimulating factor (GM-CSF), which cause phagocytic white cells of all types to differentiate and divide.

The nature of the membrane receptors for antigen on B cells and T cells is fairly well understood. Each B cell has approximately 10^5 membrane-bound antibody molecules (IgD or IgM) that correspond in specificity to the antibody the cell is programmed to produce (these receptors being referred to as BCRs). CD32 (Fc γ R II) on B cells are receptors for the Fc region of IgG. CD21 and CD35 on B cells are receptors for

complement components. Each T cell has about 10^5 molecules of a specific antigen-binding T cell receptor (a TCR) exposed on its surface. The TCR is similar, but not identical, to an antibody. There are two types of T cells that differ in their TCRs, alpha/beta ($\alpha\beta$) T cells and gamma/delta ($\gamma\delta$) T cells. The TCR of alpha/beta T cells binds a bimolecular complex displayed by a Class I MHC molecule at the surface of an antigen-presenting cell. As noted above, most Th cells express CD4, whereas most Tc cells express CD8.

Both BCRs and TCRs are similar in that they are integral membrane proteins, they are present in thousands of identical copies exposed at the cell surface, they are made before the cell ever encounters an antigen, they are encoded by genes assembled by the recombination of segments of DNA, they have a unique binding site that binds through non-covalent forces to a portion of the antigen called an epitope (or antigenic determinant) that depends on complementarity of the surface of the receptor and the surface of the epitope, and successful binding of the antigen receptor to the epitope, if accompanied by additional signals, results in stimulation of the cell to leave G_0 and enter the cell cycle and repeated mitosis that leads to the development of a clone of cells bearing the same antigen receptor, *i.e.*, a clone of cells of the identical specificity. BCRs and TCRs differ in their structure, the genes that encode them, and the type of epitope to which they bind.

Induction of a primary immune response begins when an antigen penetrates epithelial surfaces. It will eventually come into contact with macrophages or certain other classes of antigen presenting cells, including B cells, monocytes, dendritic cells, Langerhans cells, and endothelial cells. Antigens, such as bacterial cells, are internalized by endocytosis and “processed” by APCs, then “presented” to immunocompetent lymphocytes to initiate the early steps of the immunological response. Processing by a macrophage (for example) results in attaching antigenic materials to the surface of the membrane in association with Class II MHC molecules on the surface of the cell. The antigen-class II MHC complex is presented to a T-helper (Th2) cell which is able to recognize processed antigen associated with a Class II MHC molecule on the membrane of the macrophage. This interaction, together with stimulation by IL-1 from secreted by the macrophage, will activate the Th2 cell.

As indicated above, B cells themselves behave as APCs. Cross-linked antigens bound to antibody receptors on the surface of a B cell cause internalization of some of the antigen and expression on the B cell membrane together with Class II MHC molecules. The Th2 cell recognizes the antigen together with the Class II MHC molecules, and secretes the various lymphokines that activate the B cells to become antibody-secreting plasma cells and memory B cells. Even if the antigen cannot cross-link the receptor, it may be endocytosed by the B cell, processed, and returned to the surface in association with Class II MHC where it can be recognized by specific Th2 cells which will become activated to initiate B cell differentiation and proliferation. In any case, the overall B cell response leads to antibody-mediated immunity.

The antigen receptors on B cell surfaces are thought to be the specific types of antibodies that they are genetically-programmed to produce. Hence, there are thousands of sub-populations of B cells distinguished by their ability to produce a unique antibody molecule. B cells can also react with a homologous antigen on the surface of the macrophage, or with soluble antigens. When a B cell is bound to antigen, and simultaneously is stimulated by IL-4 produced by a nearby Th2 cell, the B cell is stimulated to grow and divide to form a clone of identical B cells, each capable of producing identical antibody molecules. The activated B cells further differentiate into plasma cells which synthesize and secrete large amounts of antibody, and into memory B cells. The antibodies produced and secreted by the plasma cells will react specifically with the homologous antigen that induced their formation. Many of these reactions lead to host defense and to prevention of reinfection by pathogens. Memory cells play a role in secondary immune responses. Plasma cells are relatively short-lived (about one week) but produce large amounts of antibody during this period. Memory cells, on the other hand, are relatively long-lived and upon subsequent exposure to antigen they become quickly transformed into antibody-producing plasma cells.

Generation of cell mediated immunity begins when, for example, a cytotoxic T cell recognizes a processed antigen associated with Class I MHC on the membrane of a cell (usually an altered self cell, but possibly a transplanted tissue cell, or a eukaryotic parasite). Under stimulation by IL-2 produced by Th2 cells, the Tc cell becomes activated

to become a cytotoxic T lymphocyte capable of lysing the cell which is showing the new foreign antigen on its surface, a primary manifestation of cell-mediated immunity. The interaction between an antigen-presenting macrophage and a Th cell stimulates the macrophage to produce and secrete a Interleukin-1 that acts locally on the Th cell, stimulating the Th-cell to differentiate and produce its own cytokines (which may here be called lymphokines because they arise from a lymphocyte). These lymphokines have various functions. IL-4 has an immediate effect on nearby B cells. IL-2 has an immediate effect on T cells as described above.

Leucocytes also express adhesion promoting receptors that mediate cell-cell and cell-matrix interactions. These adhesive interactions are crucial to the regulation of haemopoiesis and thymocyte maturation, the direction and control of leucocyte traffic and migration through tissues, and the development of immune and non-immune inflammatory responses. Several families of adhesion receptors have been identified. The leucocyte integrin family comprises three alpha-beta heterodimeric membrane glycoproteins that share a common beta subunit, designated CD18. The alpha subunits of each of the three members, lymphocyte function associated antigen-1 (LFA-1), macrophage antigen-1 (Mac-1) and p150,95 are designated CD11a, b, and c respectively. These adhesion molecules play a critical part in the immune and inflammatory responses of leucocytes. The leucocyte integrin family is, in turn, part of the integrin superfamily, members of which are evolutionally, structurally and functionally related. Another Integrin subfamily found on leucocytes is the VLA group, so-called because the "very late activation antigens" VLA-1 and VLA-2 were originally found to appear late in T-cell activation. Members of this family function mainly as extracellular matrix adhesion receptors and are found both on haemopoietic and non-haemopoietic cells. They play a part in diverse cellular functions including tissue organisation, lymphocyte recirculation and T-cell immune responses. Another integrin subfamily, the cytoadhesins, are receptors on platelets and endothelial cells that bind extracellular matrix proteins. A second family of adhesion receptors is the immunoglobulin superfamily, members of which include CD2, LFA-3, and ICAM-1, which participate in T-cell adhesive interactions, and the antigen-specific receptors of T and B cells, CD4, CD8, and the MHC Class I and II molecules. Another recognized family of

adhesion receptors is the selectins, characterized by a common lectin domain. Leucocyte adhesion molecule-1 (LAM-1), which is the human homologue of the murine homing receptor, MEL-14, is expressed on leucocytes, while endothelial leucocyte adhesion molecule-1 (ELAM-1) and granule membrane protein (GMP-140) are expressed on
5 stimulated endothelial cells and activated platelets.

Activation of an immune response requires physical cell-cell contact in addition to cytokines. Thus, for example, development of B and T cell precursors require intimate contact with stromal cells. At least three critical cell-cell contact events are required for the generation of immune responses. The first is initial contact of a specific
10 antigen with a naive T cell. Because of the requirement for MHC presentation, this is an obligate cell contact event. In normal situations the critical antigen presenting cell is the dendritic cell. In addition to the MHC/peptide-TCR interaction there are other non-antigen specific membrane bound ligand-receptor pairs which are important for the dendritic cell-T cell interaction. The principal one is the association of the CD28 molecule on the T cell
15 with either of two ligands, B7.1 (CD80) and B7.2 (CD86), on the dendritic cell. These molecules are termed accessory molecules and it is understood that the CD28 molecule delivers an essential second signal to the T cell without which the T cell does not become activated.

A second essential cell-cell contact is between the activated T cell and an
20 antigen-specific B cell. Most antigens are T cell-dependent, that is, an antibody response to the antigen absolutely requires T cell help. This help is delivered both by cytokines and by cell-cell contact. Cells bind specific antigen via surface Ig, then internalize, process, and present it on Class II MHC molecules. This enables them to be recognized by T cells specific for helper epitopes from the specific antigen. This cell-cell interaction also requires
25 CD28 binding to B7 on the B cell. In addition, a molecule called CD40 ligand or CD154, the expression of which is induced upon T cell activation, binds to CD40 on B cells. CD40 crosslinking promotes B cell proliferation, prevents apoptosis of germinal-center B cells, and promotes immunoglobulin isotype switching. The CD28-B7 and CD40-CD40L receptor ligand interactions are both essential for the dialogue between B and T cells that
30 causes their mutual activation.

A third cell-cell interaction that is essential in immune responses is the binding of activated B cells (which have migrated into a specialised structure in lymphoid organs called germinal centers) to follicular dendritic cells (FDCs). FDCs are specialized stromal cells that hold intact, *i.e.*, unprocessed, antigen on their surface in the form of long-lived immune complexes. Among other molecules, FDCs express CD23, which binds to germinal center B cells via a CR2 receptor and stimulates differentiation to plasma cells. Time is required before a primary immune response is effective as a host defense. Antigens have to be recognized, taken up, digested, processed, and presented by APCs. A few select Th cells must react with antigen and respond; preexisting B or T lymphocytes must encounter the antigen and proliferate and differentiate into effector cells (plasma cells or Tc cells). In the case of antibody-mediated immunity, antibody level has to build up to an effective physiological concentration to render its host resistant. It may take several days or weeks to reach a level of effective immunity, even though this immunity may persist for many months, or years, or even a lifetime, due to the presence of the antibodies. In natural infections, the inoculum is small, and even though the antigenic stimulus increases during microbial replication, only small amounts of antibody are formed within the first few days, and circulating antibody is not detectable until about a week after infection.

With regard to induction of a secondary immune response, it is understood that on re-exposure to microbial antigens (secondary exposure to antigen), there is an accelerated immunological response, *i.e.*, the secondary or memory response. Larger amounts of antibodies are formed in only 1-2 days. This is due to the activities of specific memory B cells or memory T cells which were formed during the primary immune response. These memory cells, when stimulated by homologous antigen, “remember” having previously seen the antigen, and are able to rapidly divide and differentiate into effector cells. Stimulating memory cells to rapidly produce very high (effective) levels of persistent circulating antibodies is the basis for giving “booster”-type vaccinations to humans and pets. Thus, following the first exposure to an antigen the immune response (as evidenced by following the concentration of specific antibody in the serum) develops gradually over a period of days, reaches a low plateau within 2-3 weeks, and usually begins to decline in a relatively short period of time. When the antigen is encountered a second

time, a secondary (memory) response causes a rapid rise in the concentration of antibody, reaching a much higher level in the serum, which may persist for a relatively long period of time. This is not to say that a protective level of antibody may not be reached by primary exposure alone, but usually to ensure a high level of protective antibody that persists over a long period of time, it is necessary to have repeated antigenic stimulation of the immune system.

An immunoglobulin molecule (abbreviated Ig), is a multimeric protein composed of two identical light chain polypeptides and two identical heavy chain polypeptides (H_2L_2) that are joined into a macromolecular complex by interchain disulfide bonds, *i.e.*, covalent bonds between the sulfhydryl groups of neighboring cysteine residues. There are various classes of human antibody proteins, each of which is produced by a specific clone of plasma cells. Five human immunoglobulin classes are defined on the basis of their heavy chain composition, and are named IgG, IgM, IgA, IgE, and IgD. The IgG-class and IgA-class antibodies are further divided into subclasses, namely, IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2. Intrachain disulfide bonds join different areas of the same polypeptide chain, which results in the formation of loops that, along with adjacent amino acids, constitute the immunoglobulin domains. At the amino-terminal portion (also called the "NH₂-terminus" or the "N-terminus"), each light chain and each heavy chain has a single variable region that shows considerable variation in amino acid composition from one antibody to another. The light chain variable region, V_L , associates with the variable region of a heavy chain, V_H , to form the antigen binding site of the immunoglobulin, called the Fv.

In addition to variable regions, each of the antibody chains have one or more constant regions. Light chains have a single constant region domain. Thus, light chains have one variable region and one constant region. Heavy chains have several constant region domains. The heavy chains in IgG, IgA, and IgD antibodies have three constant region domains, which are designated CH1, CH2, and CH3, and the heavy chains in IgM and IgE antibodies have four constant region domains, CH1, CH2, CH3 and CH4. Thus, heavy chains have one variable region and three or four constant regions. Immunoglobulin structure and function are reviewed, for example, in Harlow *et al.*, Eds., *Antibodies: A*

Laboratory Manual, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988).

The heavy chains of immunoglobulins can also be divided into three functional regions: the Fd region (a fragment comprising V_H and CH1, *i.e.*, the two N-terminal domains of the heavy chain), the hinge region, and the Fc region (the "fragment crystallizable" region, derived from constant regions and formed after pepsin digestion). The Fd region in combination with the light chain forms an Fab (the "fragment antigen-binding"). Because an antigen will react stereochemically with the antigen-binding region at the amino terminus of each Fab the IgG molecule is divalent, *i.e.*, it can bind to two antigen molecules. The Fc contains the domains that interact with immunoglobulin receptors on cells and with the initial elements of the complement cascade. Thus, the Fc fragment is generally considered responsible for the effector functions of an immunoglobulin, such as complement fixation and binding to Fc receptors. Pepsin sometimes also cleaves before the third constant domain (CH3) of the heavy chain to give a large fragment F(abc) and a small fragment pFcb. These terms are also used for analogous regions of the other immunoglobulins. The hinge region, found in IgG, IgA, and IgD class antibodies, acts as a flexible spacer, allowing the Fab portion to move freely in space. In contrast to the constant regions, the hinge domains are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses.

For example, the length and flexibility of the hinge region varies among the IgG subclasses. The hinge region of IgG1 encompasses amino acids 216-231 and because it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges. IgG2 has a shorter hinge than IgG1, with 12 amino acid residues and four disulfide bridges. The hinge region of IgG2 lacks a glycine residue, it is relatively short and contains a rigid poly-proline double helix, stabilised by extra inter-heavy chain disulfide bridges. These properties restrict the flexibility of the IgG2 molecule. IgG3 differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG1 hinge), containing 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix. In IgG3 the Fab fragments are relatively far away from the Fc fragment,

giving the molecule a greater flexibility. The elongated hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2. The flexibility of the hinge region reportedly decreases in the order
5 IgG3>IgG1>IgG4>IgG2. The four IgG subclasses also differ from each other with respect to their effector functions. This difference is related to differences in structure, including with respect to the interaction between the variable region, Fab fragments, and the constant Fc fragment. Nevertheless, aside from glycosylation within the CH2 region, for example, in spite of this knowledge there are no set rules or conventions regarding means or methods to
10 change features, including sequences, of these subclasses of molecule to change, control, add, or remove different functions, for example, ADCC, CDC, and other functions.

According to crystallographic studies, the immunoglobulin hinge region can be further subdivided functionally into three regions: the upper hinge region, the core region, and the lower hinge region. Shin *et al.*, 1992 *Immunological Reviews* 130:87. The upper
15 hinge region includes amino acids from the carboxyl end of CH1 to the first residue in the hinge that restricts motion, generally the first cysteine residue that forms an interchain disulfide bond between the two heavy chains. The length of the upper hinge region correlates with the segmental flexibility of the antibody. The core hinge region contains the inter-heavy chain disulfide bridges, and the lower hinge region joins the amino terminal end
20 of the CH2 domain and includes residues in CH2. *Id.* The core hinge region of human IgG1 contains the sequence Cys-Pro-Pro-Cys which, when dimerized by disulfide bond formation, results in a cyclic octapeptide believed to act as a pivot, thus conferring flexibility. The hinge region may also contain one or more glycosylation sites, which include a number of structurally distinct types of sites for carbohydrate attachment. For example, IgA1 contains
25 five glycosylation sites within a 17 amino acid segment of the hinge region, conferring resistance of the hinge region polypeptide to intestinal proteases, considered an advantageous property for a secretory immunoglobulin.

Conformational changes permitted by the structure and flexibility of the immunoglobulin hinge region polypeptide sequence may also affect the effector functions of
30 the Fc portion of the antibody. Three general categories of effector functions associated

with the Fc region include (1) activation of the classical complement cascade, (2) interaction with effector cells, and (3) compartmentalization of immunoglobulins. The different human IgG subclasses vary in the relative efficacies with which they fix complement, or activate and amplify the steps of the complement cascade. See, e.g., Kirschfink, 2001 *Immunol. Rev.* **180**:177; Chakraborti *et al.*, 2000 *Cell Signal* **12**:607; Kohl *et al.*, 1999 *Mol. Immunol.* **36**:893; Marsh *et al.*, 1999 *Curr. Opin. Nephrol. Hypertens.* **8**:557; Speth *et al.*, 1999 *Wien Klin. Wochenschr.* **111**:378.

Complement-dependent cytotoxicity (CDC) is believed to be a significant mechanism for clearance of specific target cells such as tumor cells. CDC is a stream of events that consists of a series of enzymes that become activated by each other in a cascade fashion. Complement has an important role in clearing antigen, accomplished by its four major functions: (1) local vasodilation; (2) attraction of immune cells, especially phagocytes (chemotaxis); (3) tagging of foreign organisms for phagocytosis (opsonization); and (4) destruction of invading organisms by the membrane attack complex (MAC attack). The central molecule is the C3 protein. It is an enzyme that is split into two fragments by components of either the classical pathway or the alternative pathway. The classical pathway is induced by antibodies, especially IgG and IgM, while the alternative pathway is nonspecifically stimulated by bacterial products like lipopolysaccharide (LPS). Briefly, the products of the C3 split include a small peptide C3a which is chemotactic for phagocytic immune cells and results in local vasodilation by causing the release of C5a fragment from C5. The other part of C3, C3b coats antigens on the surface of foreign organisms and acts to opsonize the organism for destruction. C3b also reacts with other components of the complement system to form an MAC consisting of C5b, C6, C7, C8 and C9.

In general, IgG1 and IgG3 most effectively fix complement, IgG2 is less effective, and IgG4 does not activate complement. Complement activation is initiated by binding of C1q, a subunit of the first component C1 in the cascade, to an antigen-antibody complex. Even though the binding site for C1q is located in the CH2 domain of the antibody, the hinge region influences the ability of the antibody to activate the cascade. For example, recombinant immunoglobulins lacking a hinge region are unable to activate complement. Shin *et al.*, 1992. Without the flexibility conferred by the hinge region, the

Fab portion of the antibody bound to the antigen may not be able to adopt the conformation required to permit C1q to bind to CH2. *See id.* Hinge length and segmental flexibility have been reported to correlate with complement activation; however, the correlation is not absolute. Human IgG3 molecules with altered hinge regions that are as rigid as IgG4, for example, can still effectively activate the cascade.

The absence of a hinge region, or a lack of a functional hinge region, can also affect the ability of certain human IgG immunoglobulins to bind Fc receptors on immune effector cells. Binding of an immunoglobulin to an Fc receptor facilitates antibody-dependent cell-mediated cytotoxicity, which as noted above is presumed to be an important mechanism for the elimination of tumor cells. The human IgG Fc receptor (FcR) family is divided into three groups, FcγRI (CD64), which is capable of binding IgG with high affinity, and FcγRII (CD32) and FcγRIII (CD16), both of which are lower affinity receptors. The molecular interaction between each of the three receptors and an immunoglobulin has not been defined precisely, but experimental evidence indicates that residues in the hinge proximal region of the CH2 domain may be important to the specificity of the interaction between the antibody and the Fc receptor. IgG1 myeloma proteins and recombinant IgG3 chimeric antibodies that lack a hinge region are reportedly unable to bind FcγRI, perhaps because accessibility to CH2 is decreased. Shin *et al.*, 1993 *Intern. Rev. Immunol.* 10:177, 178-79.

Unusual and apparently evolutionarily unrelated exceptions to the H₂L₂ structure of conventional antibodies occur in some isotypes of the immunoglobulins found in camelids (camels, dromedaries and llamas; Hamers-Casterman *et al.*, 1993 *Nature* 363:446; Nguyen *et al.*, 1998 *J. Mol. Biol.* 275:413), nurse sharks (Roux *et al.*, 1998 *Proc. Nat. Acad. Sci. USA* 95:11804), and in the spotted ratfish (Nguyen, *et al.*, "Heavy-chain antibodies in Camelidae; a case of evolutionary innovation," 2002 *Immunogenetics* 54(1):39-47). These antibodies can apparently form antigen-binding regions using only heavy chain variable region, *i.e.*, these functional antibodies are homodimers of heavy chains only (referred to as "heavy-chain antibodies" or "HCAbs"). In both species, these variable regions often contain an extended third complementarity determining region (CDR3) that may help compensate for the lack of a light chain variable region, and there are

frequent disulfide bonds between CDR regions that presumably help to stabilize the binding site. Muyldermans *et al.*, 1994 *Prot. Engineer.* 7:1129; Roux *et al.*, 1998. However, the precise function of the heavy chain-only “antibodies” is unknown, and the evolutionary pressure leading to their formation has not been identified. See, *e.g.*, Nguyen, *et al.*, 2002, *supra*. Camelids, including camels, llamas, and alpacas, also express conventional H₂L₂ antibodies, and the heavy chain-only antibodies thus do not appear to be present in these animals simply as an alternative antibody structure.

Variable regions (V_HH) of the camelid heavy chain-only immunoglobulins and conventional (H₂L₂) heavy chain variable regions contain amino acid differences, including differences at several positions that may be involved in the interface between conventional V_H and V_L domains. Nguyen *et al.*, 1998 *J. Mol. Biol* 275:413; Muyldermans *et al.*, 1994 *Prot. Engineer.* 7:1129. Camelid V_HH reportedly recombines with IgG2 and IgG3 constant regions that contain hinge, CH2, and CH3 domains and lack a CH1 domain. Hamers-Casterman *et al.*, 1993 *Nature* 363:446. Interestingly, V_HH are encoded by a chromosomal locus distinct from the V_H locus (Nguyen *et al.*, 1998, *supra*), indicating that camelid B cells have evolved complex mechanisms of antigen recognition and differentiation. Thus, for example, llama IgG1 is a conventional (H₂L₂) antibody isotype in which V_H recombines with a constant region that contains hinge, CH1, CH2 and CH3 domains, whereas the llama IgG2 and IgG3 are heavy chain-only isotypes that lack CH1 domains and that contain no light chains.

The classes of immunoglobulins have different physical and chemical characteristics and they exhibit unique biological properties. Their synthesis occurs at different stages and rates during an immune response and/or during the course of an infection. Their importance and functions in host resistance (immunity) are different.

Immunoglobulin G (IgG), a protein with a molecular weight of about 150,000 daltons (150kD), is the predominant Ig in the serum. It makes up about 80% of the total antibody found in an animal at any given time, being 75% of the total serum antibody. It can diffuse out of the blood stream into the extravascular spaces and it is the most common Ig found there. Its concentration in tissue fluids is increased during inflammation, and it is particularly effective at the neutralization of bacterial extracellular toxins and viruses. It

also has opsonizing ability and complement-fixing ability. The polypeptide composition of the Fc region of all IgG1 antibody molecules is relatively constant regardless of antibody specificity; however, as noted above, the Fab regions always differ in their exact amino acid sequences depending upon their antigenic specificity. Specific amino acid regions of the Fc portion of the molecule are recognized by receptors on phagocytes and certain other cells, and the Fc domain contains a peptide region that will bind to and activate complement, which is often required for the manifestation of antibody-mediated immunity. Because the IgG molecule is divalent, it can cross-link antigen molecules, which may lead to precipitation or agglutination of antigens; if IgG is bound to antigen on a microbial cell or surface, its Fc region may provide an extrinsic ligand that will be recognized by specific receptors on phagocytes. Microbial cells or viruses coated with IgG molecules are opsonized for phagocytosis, and opsonized pathogens are taken up and destroyed much more readily by phagocytes than their non-opsonized counterparts. IgG, as well as IgM and IgA, will neutralize the activity of toxins, including bacterial exotoxins. Furthermore, cross-linked IgG molecules on the surface of a cell can bind and activate complement from the serum and set off a cascade of reactions that can lead to destruction of the cell.

IgM is the first immunoglobulin to appear in the blood stream during the course of an infection. It is mainly confined to the bloodstream and provides protection against blood-borne pathogens. IgM makes up about 10% serum immunoglobulins, and is arranged to resemble a pentamer of five immunoglobulin molecules (having a molecular weight of about 900kD) tethered together at by their Fc domains. In addition to covalent linkages between the monomeric subunits, the pentamer is stabilized by a 15kd polypeptide called J chain. IgM, therefore, has a theoretical “valence” of ten (*i.e.*, it has ten exposed Fab domains). Probably, the most important role of IgM is its ability to function early in the immune responses against blood-borne pathogens given its efficiency in agglutinating particulate antigens. IgM binds also complement strongly and IgM antibodies bound to a microbial surface act as opsonins, rendering the microbe more susceptible to phagocytosis. In the presence of complement and IgM whole microbial cells may be killed and lysed. As noted above, IgM also appears on the surfaces of mature B cells as a transmembranous

monomer where it functions as an antigen receptor, capable of activating B cells when bound to antigen.

Gene rearrangement at the immunoglobulin loci during lymphoid development generates a repertoire of B lymphocytes that express a diversity of antigen receptors. The gene rearrangement, which is catalysed by the rearrangement-activating gene ("RAG") recombinase, integrates the immunoglobulin V, D and J gene segments to yield productively rearranged immunoglobulin genes that encode the heavy and light chains of IgM antibodies. The diversity of IgM antibodies in this primary repertoire is achieved through combinatorial mechanisms (the choice of V, D and J gene segments utilized in a particular antibody), as well as junctional diversity. The joining of V, D and J gene segments is somewhat imprecise, and nucleotides may be inserted at the junction in a non-templated manner. There is therefore a very high degree of resultant diversity at the V-D-J borders. This contributes in a major way to the structural diversity of the third complementarity determining region of the antibody, a region that often plays a critical role in antigen recognition. This primary repertoire of IgM antibodies comprises a few million different structures. The size of this repertoire means that any incoming antigen is likely to encounter an antibody that recognizes it with acceptable affinity. A high-affinity binding site is unlikely to be available for most incoming antigens (the repertoire is not large enough), but the affinity of the available IgM antibodies in the primary repertoire will vary from antigen to antigen. If an epitope is re-iterated at high density on the surface of the antigen (*e.g.*, a repeated structure on the surface of a virus or bacterium), then an IgM antibody may nevertheless be effective in mediating clearance of the organism, despite the low affinity of the individual interaction between antigenic epitope and immunoglobulin combining site. The density of the epitopes may allow multivalent interactions with IgM, leading to a high-avidity interaction, providing that a suitable spacing of antigenic epitopes can occur. Nevertheless, to ensure an effective and specific response, especially when the concentration of antigen is low (as may occur when the body is faced with a very small number of infecting viral particles), it would be preferable if high-affinity antibodies were available for neutralizing, for example, an infecting organism. The size of the primary repertoire mitigates against the likelihood of such high-affinity antibodies being present in

this repertoire. The immune system therefore operates using a two-stage strategy. The primary repertoire of IgM antibodies is generated by a process of gene rearrangement and takes place prior to antigen encounter during early lymphocyte development. However, once foreign antigen has been encountered, those B cells in the primary repertoire that encode suitable (albeit low-affinity) antibodies are selectively expanded and subjected to an iterative alternation of directed hypermutation and antigen-mediated selection. This allows a significant maturation in affinity of the antigen-specific antibodies that are produced. Antigen triggering also drives isotype switch recombination. Thus, in the absence of external antigen stimulation and any maternally derived immunoglobulin, the serum will only contain a diversity of unmutated IgM molecules that have been generated by gene rearrangement. This repertoire shifts with age as a result of continuous antigen exposure, such that the majority of the serum immunoglobulin in older animals is composed of mutated IgG (and IgA) molecules whose specificities have developed as a consequence of antigen selection.

IgA exists as a H_2L_2 monomer of about 160kD in serum and, in secretions, as a dimer of the H_2L_2 monomer of about 400kD. As with IgM, polymerization (dimerization) is via a J-chain. IgA has two subclasses based on different heavy chains, IgA1 and IgA2. IgA1 is produced in bone marrow and makes up most of the serum IgA. Both IgA1 and IgA2 are synthesized in GALT (gut associated lymphoid tissues) to be secreted onto the mucosal surfaces. Because IgA may be synthesized locally and secreted in the seromucous secretions of the body, it is sometimes referred to as secretory antibody or sIgA. Quantitatively, IgA is synthesized in amounts greater than IgG, but it has a short half life in serum (6 days), and it is lost in secretory products. The concentration of IgA in serum is about 15% of the total antibody. Secretion of dimeric IgA is mediated by a 100kD glycoprotein called secretory component. It is the addition of the secretory piece to IgA molecules that accounts for their ability to exit the body to mucosal surfaces via the exocrine glands. IgM can be transported similarly and makes up a small proportion of secretory antibodies. Secretory IgA is the predominant immunoglobulin present in gastrointestinal fluids, nasal secretions, saliva, tears and other mucous secretions of the body. IgA antibodies are important in resistance to infection of the mucosal surfaces of the body,

particularly the respiratory, intestinal and urogenital tracts. IgA acts as a protective coating for the mucous surfaces against microbial adherence or initial colonization. It can also neutralize toxin activity on mucosal surfaces. Fc receptors for IgA-coated microorganisms found on monocytes and neutrophils derived from the respiratory mucosa suggest that IgA may have a role in the lung, at least, in opsonization of pathogens. Secretory IgA is also transferred via the milk, *i.e.*, the colostrum, from a nursing mother to a newborn, which provides passive immunity to many pathogens, especially those that enter by way of the GI tract.

IgE is a immunoglobulin of about 190kD that accounts for about 0.002% of the total serum immunoglobulins. It is produced by plasma cells below the respiratory and intestinal epithelia. The majority of IgE is bound to tissue cells, especially mast cells. If an infectious agent succeeds in penetrating the IgA barrier, it comes up against the next line of defense, the MALT (mucosa-associated lymphoid tissues) system which is managed by IgE. IgE is bound very firmly to specific IgE Fc receptors on mast cells. Contact with antigen leads to release of mediators of inflammation from the mast cells, which effectively recruits various agents of the immune response including complement, chemotactic factors for phagocytes, T cells, *etc.* Although a well-known manifestation of this reaction is a type of immediate hypersensitivity reaction called atopic allergy (*e.g.*, hives, asthma, hay fever, *etc.*), the MALT responses act as a defense mechanism because they amplify the inflammatory response and may facilitate rejection of a pathogen.

IgD is a molecule of about 175kd that resembles IgG in its monomeric form. IgD antibodies are found for the most part on the surfaces of B lymphocytes. The same cells may also carry IgM antibody. As noted above, it is thought that IgD and IgM function as mutually-interacting antigen receptors for control of B cell activation and suppression. Hence, IgD may have an immunoregulatory function.

In addition to opsonization, activation of complement, and ADCC, antibodies have other functions in host defense including steric hindrance, toxin neutralization, agglutination, and precipitation. With regard to steric hindrance, it is understood that antibodies combine with the surfaces of microorganisms and may block or prevent their attachment to susceptible cells or mucosal surfaces. Antibody against a viral component can

block attachment of the virus to susceptible host cells and thereby reduce infectivity. Secretory IgA can block attachment of pathogens to mucosal surfaces. Toxin-neutralizing antibodies (antitoxins) can also react with a soluble bacterial toxin and block the interaction of the toxin with its specific target cell or substrate. Antibodies can also combine with the surfaces of microorganisms or soluble antigens and cause them to agglutinate or precipitate. This reduces the number of separate infectious units and makes them more readily phagocytosed because the clump of particles is larger in size. Floccules or aggregates of neutralized toxin may be removed by phagocytes.

Antibodies have been proposed for use in therapy. Animals, including humans and mice have the ability to make antibodies able to recognize (by binding to) virtually any antigenic determinant and to discriminate between similar epitopes. Not only does this provide the basis for protection against disease organisms, but it makes antibodies attractive candidates to target other types of molecules found in the body, such as receptors or other proteins present on the surface of normal cells and molecules present uniquely on the surface of cancer cells. Thus the remarkable specificity of antibodies makes them promising agents for human therapy.

Initial antibody preparations available for use, such as intravenous gammaglobulins, included animal and human antisera that were used *in vivo* to destroy bacteria (tetanus, pneumococcus) and neutralize virus (hepatitis A and B, rabies, cytomegalovirus, and varicella zoster) in the blood of infected individuals. Possibly the most important early application was the use and endotoxins. However, there are problems associated with the use of antibodies in human therapy because the response of the immune system to any antigen, even the simplest, is "polyclonal," *i.e.*, the system manufactures antibodies of a great range of structures both in their binding regions as well as in their effector regions. Polyclonal antibody treatment was also associated with unwanted side effects. In addition to the polyclonal nature of these antibody preparations, there was the risk of infection from contaminating viruses. Serum sickness, anaphylaxis, and were also considered limiting factors. Furthermore, even if one were to isolate a single antibody-secreting cell, and place it in culture, it would die out after a few generations because of the limited growth potential of all normal somatic cells.

Until the late 1970s, polyclonal antibodies obtained from the blood serum of immunized animals, provided the only source of antibodies for research or treatment of disease. Isolation of specific antibodies was essentially impossible until Kohler and Milstein discovered how to make "monoclonal antibodies" that would have a single specificity, that
5 would all be alike due to manufacture by a single clone of plasma cells, and that could be grown indefinitely. This technique was described in a 1975 publication (*Nature* 256:495-97), and Köhler and Milstein received the 1984 Nobel Prize in Medicine for their work.

The first step in Kohler and Milstein's technique for production of monoclonal antibodies involves immunizing an experimental animal with the antigen of
10 interest. In most of their experiments, Kohler and Milstein injected a mouse with sheep red blood cells. The mouse's body initiates an immune response and begins producing antibodies specific to the antigen. The mouse's spleen is then removed and B cells producing the antibody of interest are isolated. Tumor-producing cells that have been grown in culture are then fused with the B lymphocytes using polyethylene glycol in order to
15 produce a "hybridoma." Only hybridomas resulting from the fusion will survive. The spleen lymphocyte has a limited life span, so any B cells that did not fuse with a myeloma will die in the culture. As well, those cells that lack the antibody-producing aspect of the B cell will not secrete the enzyme HGPRT, which is required for growth in the HAT medium. The hypoxanthine-aminopterinthymidine (HAT) medium, on which the cells are grown,
20 inhibits the pathway for nucleotide synthesis. Cells which produce HGPRT can bypass this pathway and continue to grow. By placing the fused cells in a HAT medium, the true hybridomas can be isolated (McKay, Raff, Reichardt 1981). The isolated hybridoma cells are then screened for specificity to the desired antigen. Because each hybridoma descends from one B cell, it makes copies of only one antibody. The hybridoma that produces the
25 antibody of interest is grown in culture to produce large amounts of monoclonal antibodies, which are then isolated for further use. The technique is called somatic cell hybridization, and the resulting hybridoma (selected for both immortality and production of the specific antibody of interest) may be cultured indefinitely, *i.e.*, it is a potentially immortal cell line.

Monoclonal antibodies are now widely used as diagnostic and research
30 reagents. However, their introduction into human therapy has been much slower. One

principal difficulty is that mouse antibodies are “seen” by the human immune system as foreign, and the human patient mounts an immune response against them, producing HAMA (“human anti-mouse antibodies”). These not only cause the therapeutic antibodies to be quickly eliminated from the host, but also form immune complexes that cause damage to the kidneys.

Two approaches have been used in an attempt to reduce the problem of HAMA. The first is the production of chimeric antibodies in which the antigen-binding part (variable regions) of a mouse monoclonal antibody is fused to the effector part (constant region) of a human antibody using genetic engineering. In a second approach, rodent antibodies have been altered through a technique known as complementarity determining region (CDR) grafting or “humanization.” In this process, the antigen binding sites, which are formed by three CDRs of the heavy chain and three CDRs of the light chain, are excised from cells secreting rodent mAb and grafted into the DNA coding for the framework of the human antibody. Because only the antigen-binding site CDRs, rather than the entire variable domain of the rodent antibody are transplanted, the resulting humanized antibody (a second generation or “hyperchimeric” antibody) is reportedly less immunogenic than a first generation chimeric antibody. This process has been further improved to include changes referred to as “reshaping” (Verhoeven, *et al.*, “Reshaping human antibodies: grafting an anti-lysozyme activity,” 1988 *Science* **239**:1534-1536; Riechmann, *et al.*, “Reshaping human antibodies for therapy,” 1988 *Nature* **332**:323-337; Tempest, *et al.*, “Reshaping human monoclonal antibody to inhibit respiratory syncytial virus infection in vivo,” *Bio/Technol* 1991 **9**:266-271), “hyperchimerization” (Queen, *et al.*, “A humanized antibody that binds to the human interleukin 2 receptor,” 1989 *Proc Natl Acad Sci USA* **86**:10029-10033; Co, *et al.*, “Humanized antibodies for antiviral therapy,” 1991 *Proc Natl Acad Sci USA* **88**:2869–2873; Co, *et al.*, “Chimeric and humanized antibodies with specificity for the CD33 antigen,” 1992 *J Immunol* **148**:1149-1154), and “veneering” (Mark, *et al.*, “Derivation of therapeutically active humanized and veneered anti-CD18 antibodies. In: Metcalf BW, Dalton BJ, eds. Cellular adhesion: molecular definition to therapeutic potential. New York: Plenum Press, 1994:291-312). In the reshaping process on the basis of homology, the rodent variable region is compared with the consensus sequence of the protein sequence subgroup to which

it belongs. Similarly, the selected human constant region accepting framework is compared with its family consensus sequence. Gussowal, *et al.*, "Humanization of monoclonal antibodies," 1991 *Meth Enzymol* 203:99-121. The sequence analyses identify residues, which may have undergone mutation during the affinity maturation procedure and may therefore be idiosyncratic. Inclusion of the more common human residues is said to lessen immunogenicity problems by replacing human acceptor idiosyncratic residues. Further, the reshaping process is said to allow comparison of human and rodent consensus sequences to identify any systematic "species" differences. RSV19 antibodies were humanized by employing this procedure. Taylor *et al.*, "Humanized monoclonal antibody to respiratory syncytial virus," 1991 *Lancet* 337:1411-1412; Tempest, *et al.*, "Reshaping a human monoclonal antibody to inhibit human respiratory syncytial virus infection in vivo," 1991 *Bio/Technol* 9:266-271. Hyperchimerization is an alternative method of identifying residues outside CDR regions that are likely to be involved in the reconstitution of binding activity. In this method, the human sequences are compared with murine variable region sequences and the one with highest homology is selected as the acceptor framework. As in the reshaping procedure, the "idiosyncratic" residues are replaced by more commonly occurring human residues. The non-CDR residues that may be interacting with the CDR sequences are identified. Finally, it is determined which one of these residues is to be included in the variable region framework. Humanized antibodies against CD33 antigen were reportedly developed by this method. Co, *et al.*, "Chimeric and humanized antibodies with specificity for the CD33 antigen," 1992 *J Immunol* 148:1149-154. See also Carter, *et al.*, "Humanization of an anti-p185 HER2 antibody for human cancer therapy," 1992 *Proc Natl Acad Sci USA* 89:4285-4289. The displayed surface of the protein is the primary determinant of its immunogenicity. A humanized murine antibody can thus be made less immunogenic by replacing exposed residues that differ from those commonly found in human antibodies. This method of humanization is referred to as "veneering." Appropriate replacement of the outer residues may have little or no impact on the inner domains or interdomain framework.. Veneering is a two-step process. In the first step, the most homologous human variable regions are selected and compared by each single residue to the corresponding mouse variable regions. In the second step, the mouse framework residues,

which differ from its human homologue, are replaced by the residues present in the human homologue. This replacement involves only those residues that are on the surface and at least partially exposed.

Nevertheless, it took more than a quarter century of research for monoclonal antibody technology and genetic engineering methods to result in the development of immunoglobulin molecules for treatment of human diseases. Indeed, it was not until the past five years that monoclonal antibodies became as an expanding class of therapeutics. See Glennie MJ and van de Winkel JG, *Drug Discov Today* 2003 Jun 1;8(11):503-10; Souriau C and Hudson PJ, "Recombinant antibodies for cancer diagnosis and therapy," 2003 *Expert Opin Biol Ther.* 3(2):305-18. See also Pendley C, *et al.*, "Immunogenicity of therapeutic monoclonal antibodies," 2003 *Curr Opin Mol Ther.* 5(2):172-9.

All the same, an average of less than one therapeutic antibody per year has been introduced to the market beginning in 1986, eleven years after the publication of monoclonal antibodies. Five murine monoclonal antibodies were introduced into human medicine over a ten year period from 1986-1995, including "muromonab-CD3" (OrthoClone OKT3®), which binds to a molecule on the surface of T cells and was launched in 1986 to prevent acute rejection of organ transplants; "edrecolomab" (Panorex®), launched in 1995 for treatment of colorectal cancer; "odulimomab" (Antilfa®), launched in 1997 for use in transplant rejection; and, "ibritumomab" (Zevalin® yiuixetan), launched in 2002 for use in non-Hodgkin's lymphoma. Additionally, one monoclonal Fab, "abciximab" (ReoPro®), was launched in 1995. It inhibits the clumping of platelets by binding the receptors on their surface that normally are linked by fibrinogen and may be helpful in preventing reclogging of the coronary arteries in patients who have undergone angioplasty. Three chimeric monoclonal antibodies were also launched: "rituximab" (Rituxan®), in 1997, which binds to the CD20 molecule found on most B cells and is used to treat B cell lymphomas; "basiliximab" (Simulect®), in 1998 for transplant rejection; and "infliximab" (Remicade®) which binds to tumor necrosis factor-alpha (TNF- α), in 1998 for treatment of as rheumatoid arthritis and Crohn's disease. Additionally, "abciximab" (ReoPro®), a 47.6 kD Fab fragment of the chimeric human-murine monoclonal antibody 7E3 that binds to the glycoprotein (GP) IIb/IIIa receptor of human platelets, was launched in 1995 as an adjunct

to percutaneous coronary intervention for the prevention of cardiac ischemic complications in patients undergoing percutaneous coronary intervention. Finally, seven “humanized” monoclonals were launched from 1997-2003: “daclizumab” (Zenapax®) in 1997, which binds to part of the IL-2 receptor produced at the surface of activated T cells and is used to prevent acute rejection of transplanted kidneys; “palivizumab” (Synagis®) in 1998 for RSV; “trastuzumab” (Herceptin®) in 1998, which binds HER-2, a growth factor receptor found on breast cancers cells; “gemtuzumab” (Mylotarg®) in 2000, which is a conjugate of a monoclonal antibody that binds CD33, a cell-surface molecule expressed by the cancerous cells in acute myelogenous leukemia (AML) but not found on the normal stem cells needed to repopulate the bone marrow; and “alemtuzumab” (MabCampath®) in 2001, which binds to CD52, a molecule found on white blood cells and has produced temporary remission of chronic lymphocytic leukemia; “adalimumab” (Humira® (D2E7)), a human anti-TNF monoclonal containing human-derived heavy chain and light chain variable regions and human IgG:κ constant regions was launched in 2002 for the treatment of rheumatoid arthritis; and, “omalizumab” (Xolair®), which binds to IgE and prevents it from binding to mast cells was approved in 2003 for the treatment of adults and adolescents over 12 years of age with moderate to severe persistent asthma who have a positive skin test or *in vitro* reactivity to a perennial aeroallergen and whose symptoms are inadequately controlled with inhaled corticosteroids.

Thus, protein engineering has been applied in an effort to diminish problems related to immunogenicity of administered recombinant immunoglobulin polypeptides and to try to alter antibody effector functions. However, problems remain. For example, the majority of cancer patients treated with rituximab relapse, generally within about 6-12 months, and fatal infusion reactions within 24 hours of rituximab infusion have been reported. These fatal reactions followed an infusion reaction complex that included hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation or cardiogenic shock. Acute renal failure requiring dialysis with instances of fatal outcome has also been reported in the setting of tumor lysis syndrome following treatment with rituximab, as have severe mucocutaneous reactions, some with fatal outcome. Additionally, high doses of rituximab are required for intravenous injection

because the molecule is large, approximately 150 kDa, and diffusion is limited into the lymphoid tissues where many tumor cells reside.

Trastuzumab administration can result in the development of ventricular dysfunction and congestive heart failure, and the incidence and severity of cardiac
5 dysfunction has been reported to be particularly high in patients who received trastuzumab in combination with anthracyclines and cyclophosphamide. Trastuzumab administration can also result in severe hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events.

Patients receiving daclizumab immunosuppressive therapy are at increased
10 risk for developing lymphoproliferative disorders and opportunistic infections, and it is not known whether daclizumab use will have a long-term effect on the ability of the immune system to respond to antigens first encountered during daclizumab-induced immunosuppression.

Hepatotoxicity, including severe hepatic veno-occlusive disease (VOD), has
15 also been reported in association with the use of gemtuzumab as a single agent, as part of a combination chemotherapy regimen, and in patients without a history of liver disease or hematopoietic stem-cell transplant (HSCT). Patients who receive gemtuzumab either before or after HSCT, patients with underlying hepatic disease or abnormal liver function, and patients receiving gemtuzumab in combinations with other chemotherapy may be at
20 increased risk for developing severe VOD. Death from liver failure and from VOD has been reported in patients who received gemtuzumab, and it has been cautioned that even careful monitoring may not identify all patients at risk or prevent the complications of hepatotoxicity.

Hepatotoxicity was also reported in patients receiving alemtuzumab. Serious
25 and, in some rare instances fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia have occurred in patients receiving alemtuzumab therapy. Alemtuzumab can also result in serious infusion reactions as well as opportunistic infections.

In patients treated with adalimumab, serious infections and sepsis, including
30 fatalities, have been reported, as has the exacerbation of clinical symptoms and/or

radiographic evidence of demyelinating disease, and patients treated with adalimumab in clinical trials had a higher incidence of lymphoma than the expected rate in the general population.

Serious adverse reactions in clinical studies with omalizumab have included malignancies and anaphylaxis, in which the observed incidence of malignancy among omalizumab-treated patients (0.5%) was numerically higher than among patients in control groups (0.2%).

Smaller immunoglobulin molecules have been constructed in an effort to overcome various problems associated with whole immunoglobulin therapy. Single chain immunoglobulin variable region fragment polypeptides (scFvs) are made of an immunoglobulin heavy chain variable domain joined via a short linker peptide to an immunoglobulin light chain variable domain. Huston *et al.*, 1988*Proc. Natl. Acad. Sci. USA*, **85**:5879-83. It has been suggested that the smaller size of scFv molecules may lead to more rapid clearance from plasma and more effective penetration into tissues than whole immunoglobulins. See, e.g., Jain, 1990 *Cancer Res.* **50**:814s-819s. An anti-tumor scFv was reported to show more rapid tumor penetration and more even distribution through the tumor mass than the corresponding chimeric antibody. Yokota *et al.*, *Cancer Res.* **52**:3402-08 (1992).

Despite advantages that scFv molecules may have with regard to serotherapy, drawbacks to this therapeutic approach also exist. For example, rapid clearance of scFv may prevent delivery of a minimum effective dose to the target tissue. Additionally, manufacturing adequate amounts of scFv for administration to patients has been challenging due to difficulties in expression and isolation of scFv that adversely affect yields. During expression, scFv molecules lack stability and often aggregate due to pairing of variable regions from different molecules. Furthermore, production levels of scFv molecules in mammalian expression systems are reportedly low, which may limit the potential for efficient manufacturing of scFv molecules for therapy. Davis *et al.*, 1990 *J. Biol. Chem.* **265**:10410-18; Traunecker *et al.*, 1991 *EMBO J.* **10**:3655-59. Strategies for means to improve production have been explored, and reportedly include the addition of glycosylation sites to variable regions. See, e.g., U.S. Patent No. 5,888,773; Jost *et al.*, 1994 *J. Biol.*

Chem. **269**:26267-73. Another disadvantage to the use of scFvs for therapy is the lack of effector function. An scFv that lacks the cytolytic functions, ADCC, and complement dependent-cytotoxicity may be less effective or ineffective for treating disease. Even though development of scFv technology began over 12 years ago, there are currently no scFv products approved for therapy.

Alternatively, it has been proposed that fusion of an scFv to another molecule, such as a toxin, could take advantage of the specific antigen-binding activity and the small size of an scFv to deliver the toxin to a target tissue. Chaudary *et al.*, 1989 *Nature* **339**:394; Batra *et al.*, 1991 *Mol. Cell. Biol.* **11**:2200. Conjugation or fusion of toxins to scFvs has thus been offered as an alternative strategy to provide potent, antigen-specific molecules, but dosing with such conjugates or chimeras can be limited by excessive and/or non-specific toxicity due to the toxin moiety of such preparations. Toxic effects may include supraphysiological elevation of liver enzymes and vascular leak syndrome, and other undesired effects. In addition, immunotoxins are themselves highly immunogenic upon administration to a host, and host antibodies generated against the immunotoxin limit potential usefulness for repeated therapeutic treatments of an individual.

Fusion proteins in which immunoglobulin constant region polypeptide sequences are present and nonimmunoglobulin sequences are substituted for the antibody variable regions have also been investigated. For example, CD4, the T cell surface protein recognized by HIV, was recombinantly fused to an immunoglobulin Fc effector domain, and an IL-2-IgG1 fusion protein reportedly effected complement-mediated lysis of IL-2 receptor-bearing cells. See Sensel *et al.*, *Chem. Immunol.* **65**:129-158 (1997).

An extensive introduction as well as detailed information about all aspects of recombinant antibody technology can be found in the textbook "Recombinant Antibodies" (John Wiley & Sons, NY, 1999). A comprehensive collection of detailed antibody engineering lab Protocols can be found in R. Kontermann and S. Dübel (eds.), "The Antibody Engineering Lab Manual" (Springer Verlag, Heidelberg/New York, 2000). Diseases and disorders thought to be amenable to some type of immunoglobulin therapy include cancer and immune system disorders. Cancer includes a broad range of diseases, affecting approximately one in four individuals worldwide. Rapid and unregulated

proliferation of malignant cells is a hallmark of many types of cancer, including hematological malignancies. Although patients with a hematologic malignant condition have benefited from advances in cancer therapy in the past two decades, Multani *et al.*, 1998 *J. Clin. Oncology* 16:3691-3710, and remission times have increased, most patients still
5 relapse and succumb to their disease. Barriers to cure with cytotoxic drugs include, for example, tumor cell resistance and the high toxicity of chemotherapy, which prevents optimal dosing in many patients.

Nevertheless, patients have been treated with immunotherapeutics that target malignant cells, *i.e.*, to antigens expressed on tumor cells.. With regard to the selection of
10 tumor cell surface antigens suitable for use as immunotherapy targets, it is preferable that such a target antigen is not expressed by normal tissues, particularly where the preservation of such tissue is important to host survival. In the case of hematologic malignancy, malignant cells express many antigens that are not expressed on the surfaces of stem cells or other essential cells. Treatment of a hematologic malignant condition using a therapeutic
15 regimen that depletes both normal and malignant cells of hematological origin has been acceptable where regeneration of normal cells from progenitors can occur after therapy has ended. Additionally, the target antigen is desirably expressed on all or virtually all clonogenic populations of tumor cells, and it is best that expression persists despite selective pressure from immunoglobulin therapy. Strategies that employ selection of a cell surface
20 idiotype (*e.g.*, a particular idiotope) as a target for therapy of B cell malignancy have been limited by the outgrowth of tumor cell variants with altered surface idiotype expression, even where the antigen exhibits a high degree of tumor selectivity. Meeker *et al.*, 1985 *N. Engl. J. Med.* 312:1658-65. The selected antigen should also traffic properly after an immunoglobulin binds to it. Shedding or internalization of a cell surface target antigen after
25 an immunoglobulin binds to the antigen may allow tumor cells to escape destruction, thus limiting the effectiveness of serotherapy. Finally, binding of an immunoglobulin to cell surface target antigens that transmit or transduce cellular activation signals may result in improved functional responses to immunotherapy in tumor cells, and can lead to growth arrest and/or apoptosis. While all of these properties are important, the triggering of

apoptosis after an immunoglobulin binds to the target antigen may also be a critical factor in achieving successful serotherapy.

Antigens that have been tested as targets for serotherapy of B and T cell malignancies include Ig idiotype (Brown *et al.*, 1989 *Blood* 73:651-61), CD19 (Hekman *et al.*, 1991 *Cancer Immunol. Immunother.* 32:364-72), Vlasveld *et al.*, 1995 *Cancer Immunol. Immunother.* 40:37-47), CD20 (Press *et al.*, 1987 *Blood* 69: 584-91), Maloney *et al.*, 1997 *J. Clin. Oncol.* 15:3266-74), CD21 (Scheinberg *et al.*, 1990 *J. Clin. Oncol.* 8:792-803), CD5 (Dillman *et al.*, 1986 *J. Biol. Respn. Mod.* 5:394-410), and CD52 (CAMPATH) (Pawson *et al.*, 1997 *J. Clin. Oncol.* 15:2667-72). Of these, greater benefit for therapy of B cell lymphomas has been obtained using molecules that target CD20. . Other targets have been limited by biological properties of the antigen. For example, surface idiotype can be altered through somatic mutation, allowing tumor cell escape. CD5, CD21, and CD19 are rapidly internalized after monoclonal antibody binding, allowing tumor cells to escape destruction unless monoclonal antibodies are conjugated with toxin molecules. CD22 is expressed on only a subset of B cell lymphomas, thereby limiting its usefulness, while CD52 is expressed on both T cells and B cells and may therefore generate counterproductive immunosuppression by depletion.

Treatment of patients with low grade or follicular B cell lymphoma using a chimeric CD20 monoclonal antibody has been reported to induce partial or complete responses in patients. McLaughlin *et al.*, 1996 *Blood* 88:90a (abstract, suppl. 1); Maloney *et al.*, 1997 *Blood* 90:2188-95. However, as noted above, tumor relapse commonly occurs within six months to one year. Further improvements in serotherapy are needed to induce more durable responses, for example, in low grade B cell lymphoma, and to allow effective treatment of high grade lymphoma and other B cell diseases.

Another approach has been to target radioisotopes to B cell lymphomas using monoclonal antibodies specific for CD20. While the effectiveness of therapy is reportedly increased, associated toxicity from the long *in vivo* half-life of the radioactive antibody increases also, sometimes requiring that the patient undergo stem cell rescue. Press *et al.*, 1993 *N. Eng. J. Med.* 329:1219-1224; Kaminski *et al.*, 1993 *N. Eng. J. Med.* 329:459-65. Monoclonal antibodies to CD20 have also been cleaved with proteases to yield F(ab')₂ or

Fab fragments prior to attachment of radioisotope. This has been reported to improve penetration of the radioisotope conjugate into the tumor and to shorten the *in vivo* half-life, thus reducing the toxicity to normal tissues. However, these molecules lack effector functions, including complement fixation and/or ADCC..

5 CD20 was the first human B cell lineage-specific surface molecule identified by a monoclonal antibody. It is a non-glycosylated, hydrophobic 35 kDa B cell transmembrane phosphoprotein that has both amino and carboxy ends situated in the cytoplasm. Einfeld *et al.*, 1988 *EMBO J.* **7**:711-17. CD20 is expressed by all normal mature B cells, but is not expressed by precursor B cells. Natural ligands for CD20 have not been
10 identified, and the function of CD20 in B cell biology is still incompletely understood.

Anti-CD20 monoclonal antibodies affect the viability and growth and growth of B cells. Clark *et al.*, 1986 *Proc. Natl. Acad. Sci. USA* **83**:4494-98. Extensive cross-linking of CD20 can induce apoptosis in B lymphoma cell lines, Shan *et al.*, 1998 *Blood* **91**:1644-52, and cross-linking of CD20 on the cell surface has been reported to
15 increase the magnitude and enhance the kinetics of signal transduction, for example, as detected by measuring tyrosine phosphorylation of cellular substrates. Deans *et al.*, 1993 *J. Immunol.* **146**:846-53. Therefore, in addition to cellular depletion by complement and ADCC mechanisms, Fc-receptor binding by CD20 monoclonal antibodies *in vivo* may promote apoptosis of malignant B cells by CD20 cross-linking, consistent with the theory
20 that effectiveness of CD20 therapy of human lymphoma in a SCID mouse model may be dependent upon Fc-receptor binding by the CD20 monoclonal antibody. Funakoshi *et al.*, 1996 *J. Immunotherapy* **19**:93-101. The presence of multiple membrane spanning domains in the CD20 polypeptide (Einfeld *et al.*, 1988 *EMBO J.* **7**:711-17; Stamenkovic *et al.*, 1988 *J. Exp. Med.* **167**:1975-80; Tedder *et al.*, 1988 *J. Immunol.* **141**:4388-4394), prevent CD20
25 internalization after antibody binding, and this was recognized as an important feature for therapy of B cell malignancies when a murine CD20 monoclonal antibody, 1F5, was injected into patients with B cell lymphoma, resulting in significant depletion of malignant cells and partial clinical responses. Press *et al.*, 1987 *Blood* **69**:584-91.

Because normal mature B cells also express CD20, normal B cells are
30 depleted by anti-CD20 antibody therapy. Reff, M.E. *et al.*, 1994 *Blood* **83**:435-445. After

treatment is completed, however, normal B cells can be regenerated from CD20 negative B cell precursors; therefore, patients treated with anti-CD20 therapy do not experience significant immunosuppression. Depletion of normal B cells may also be beneficial in diseases that involve inappropriate production of autoantibodies or other diseases where B cells may play a role. A chimeric monoclonal antibody specific for CD20, consisting of heavy and light chain variable regions of mouse origin fused to human IgG1 heavy chain and human kappa light chain constant regions, reportedly retained binding to CD20 and the ability to mediate ADCC and to fix complement. Liu *et al.*, 1987 *J. Immunol.* **139**:3521-26. The mechanism of anti-tumor activity of rituximab, discussed above, is thought to be a combination of several activities, including ADCC, complement fixation, and triggering of signals that promote apoptosis in malignant B cells, although the large size of rituximab prevents optimal diffusion of the molecule into lymphoid tissues that contain malignant B cells, thereby limiting these anti-tumor activities. Autoimmune diseases include autoimmune thyroid diseases, which include Graves' disease and Hashimoto's thyroiditis. In the United States alone, there are about 20 million people who have some form of autoimmune thyroid disease. Autoimmune thyroid disease results from the production of autoantibodies that either stimulate the thyroid to cause hyperthyroidism (Graves' disease) or destroy the thyroid to cause hypothyroidism (Hashimoto's thyroiditis). Stimulation of the thyroid is caused by autoantibodies that bind and activate the thyroid stimulating hormone (TSH) receptor. Destruction of the thyroid is caused by autoantibodies that react with other thyroid antigens. Current therapy for Graves' disease includes surgery, radioactive iodine, or antithyroid drug therapy. Radioactive iodine is widely used, since antithyroid medications have significant side effects and disease recurrence is high. Surgery is reserved for patients with large goiters or where there is a need for very rapid normalization of thyroid function. There are no therapies that target the production of autoantibodies responsible for stimulating the TSH receptor. Current therapy for Hashimoto's thyroiditis is levothyroxine sodium, and therapy is usually lifelong because of the low likelihood of remission. Suppressing therapy has been shown to shrink goiters in Hashimoto's thyroiditis, but no therapies that reduce autoantibody production to target the disease mechanism are known.

Rheumatoid arthritis (RA) is a chronic disease characterized by inflammation of the joints, leading to swelling, pain, and loss of function. RA affects an estimated 2.5 million people in the United States. RA is caused by a combination of events including an initial infection or injury, an abnormal immune response, and genetic factors. While
5 autoreactive T cells and B cells are present in RA, the detection of high levels of antibodies that collect in the joints, called rheumatoid factor, is used in the diagnosis of RA. Current therapy for RA includes many medications for managing pain and slowing the progression of the disease. No therapy has been found that can cure the disease. Medications include nonsteroidal antiinflammatory drugs (NSAIDS), and disease modifying antirheumatic drugs
10 (DMARDS). NSAIDS are useful in benign disease, but fail to prevent the progression to joint destruction and debility in severe RA. Both NSAIDS and DMARDS are associated with significant side effects. Only one new DMARD, Leflunomide, has been approved in over 10 years. Leflunomide blocks production of autoantibodies, reduces inflammation, and slows progression of RA. However, this drug also causes severe side effects including
15 nausea, diarrhea, hair loss, rash, and liver injury.

Systemic Lupus Erythematosus (SLE) is an autoimmune disease caused by recurrent injuries to blood vessels in multiple organs, including the kidney, skin, and joints. SLE is estimated to affect over 500,000 people in the United States. In patients with SLE, a faulty interaction between T cells and B cells results in the production of autoantibodies that
20 attack the cell nucleus. These include anti-double stranded DNA and anti-Sm antibodies. Autoantibodies that bind phospholipids are also found in about half of SLE patients, and are responsible for blood vessel damage and low blood counts. Immune complexes accumulate the kidneys, blood vessels, and joints of SLE patients, where they cause inflammation and tissue damage. No treatment for SLE has been found to cure the disease. NSAIDS and
25 DMARDS are used for therapy depending upon the severity of the disease. Plasmapheresis with plasma exchange to remove autoantibodies can cause temporary improvement in SLE patients. There is general agreement that autoantibodies are responsible for SLE, so new therapies that deplete the B cell lineage, allowing the immune system to reset as new B cells are generated from precursors, would offer hope for long lasting benefit in SLE patients.

Sjogren's syndrome is an autoimmune disease characterized by destruction of the body's moisture-producing glands. Sjogren's syndrome is one of the most prevalent autoimmune disorders, striking up to an estimated 4 million people in the United States. About half of people stricken with Sjogren's syndrome also have a connective tissue disease, such as RA, while the other half have primary Sjogren's syndrome with no other concurrent autoimmune disease. Autoantibodies, including anti-nuclear antibodies, rheumatoid factor, anti-fodrin, and anti-muscarinic receptor are often present in patients with Sjogren's syndrome. Conventional therapy includes corticosteroids, and additional more effective therapies would be of benefit.

Immune thrombocytopenic purpura (ITP) is caused by autoantibodies that bind to blood platelets and cause their destruction. Some cases of ITP are caused by drugs, and others are associated with infection, pregnancy, or autoimmune disease such as SLE. About half of all cases are classified as "idiopathic", meaning the cause is unknown. The treatment of ITP is determined by the severity of the symptoms. In some cases, no therapy is needed although in most cases immunosuppressive drugs, including corticosteroids or intravenous infusions of immune globulin to deplete T cells, are provided. Another treatment that usually results in an increased number of platelets is removal of the spleen, the organ that destroys antibody-coated platelets. More potent immunosuppressive drugs, including cyclosporine, cyclophosphamide, or azathioprine are used for patients with severe cases. Removal of autoantibodies by passage of patients' plasma over a Protein A column is used as a second line treatment in patients with severe disease. Additional more effective therapies are desired.

Multiple sclerosis (MS) is also an autoimmune disease. It is characterized by inflammation of the central nervous system and destruction of myelin, which insulates nerve cell fibers in the brain, spinal cord, and body. Although the cause of MS is unknown, it is widely believed that autoimmune T cells are primary contributors to the pathogenesis of the disease. However, high levels of antibodies are present in the cerebral spinal fluid of patients with MS, and some theories predict that the B cell response leading to antibody production is important for mediating the disease. No B cell depletion therapies have been studies in patients with MS, and there is no cure for MS. Current therapy is corticosteroids,

which can reduce the duration and severity of attacks, but do not affect the course of MS over time. New biotechnology interferon (IFN) therapies for MS have recently been approved but additional more effective therapies are desired.

Myasthenia Gravis (MG) is a chronic autoimmune neuromuscular disorder that is characterized by weakness of the voluntary muscle groups. MG affects about 40,000 people in the United States. MG is caused by autoantibodies that bind to acetylcholine receptors expressed at neuromuscular junctions. The autoantibodies reduce or block acetylcholine receptors, preventing the transmission of signals from nerves to muscles. There is no known cure for MG. Common treatments include immunosuppression with corticosteroids, cyclosporine, cyclophosphamide, or azathioprine. Surgical removal of the thymus is often used to blunt the autoimmune response. Plasmapheresis, used to reduce autoantibody levels in the blood, is effective in MG, but is short-lived because the production of autoantibodies continues. Plasmapheresis is usually reserved for severe muscle weakness prior to surgery. New and effective therapies would be of benefit.

Psoriasis affects approximately five million people, and is characterized by autoimmune inflammation in the skin. Psoriasis is also associated with arthritis in 30% (psoriatic arthritis). Many treatments, including steroids, UV light, retinoids, vitamin D derivatives, cyclosporine, methotrexate have been used but it is also plain that psoriasis would benefit from new and effective therapies. Scleroderma is a chronic autoimmune disease of the connective tissue that is also known as systemic sclerosis. Scleroderma is characterized by an overproduction of collagen, resulting in a thickening of the skin, and approximately 300,000 people in the United States have scleroderma, which would also benefit from new and effective therapies.

There is a clear need for improved compositions and methods to treat malignancies, including B cell malignancies and disorders including autoimmune diseases, disorders, and conditions, as well as other diseases, disorders, and conditions. The compositions and methods of the present invention described and claimed herein provide such improved compositions and methods as well as other advantages.

SUMMARY OF THE INVENTION

It is an aspect of the present invention to provide a binding domain-immunoglobulin fusion protein, comprising a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge or hinge-acting region polypeptide, which in turn is fused or otherwise connected to a region comprising one or more native or engineered constant regions from an immunoglobulin heavy chain, other than CH1, for example, the CH2 and CH3 regions of IgG and IgA, or the CH3 and CH4 regions of IgE. The binding domain-immunoglobulin fusion protein further comprises a region that comprises, consists essentially of, or consists of, a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the hinge region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide (or CH4 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE). Such binding domain-immunoglobulin fusion proteins are capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation. Such binding domain polypeptides are also capable of binding or specifically binding to a target, for example, a target antigen.

In certain embodiments, for example, the binding domain polypeptide comprises at least one immunoglobulin variable region polypeptide that is selected from a native or engineered immunoglobulin light chain variable region polypeptide and/or a native or engineered immunoglobulin heavy chain variable region polypeptide. In certain further embodiments the binding domain-immunoglobulin fusion protein comprises a native or engineered immunoglobulin heavy chain variable region polypeptide, wherein the heavy chain variable region polypeptide is an engineered human immunoglobulin heavy chain variable region polypeptide (or an engineered immunoglobulin heavy chain variable region polypeptide from a non-human species) comprising a mutation, substitution, or deletion of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108, 110, and/or 112. Mutations, substitutions, or deletions of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108,

110, and/or 112 in a heavy chain variable region may be included within a construct such as the construct corresponding to, for example, SEQ ID NO:____. In certain other further embodiments the fusion protein comprises a polypeptide having a sequence selected from SEQ ID NOS:___ or SEQ ID NO:____. In certain embodiments the immunoglobulin variable region polypeptide is derived from, for example, a human immunoglobulin, and in certain other embodiments the immunoglobulin variable region polypeptide comprises a humanized immunoglobulin polypeptide sequence. In certain embodiments the immunoglobulin variable region polypeptide, whether or not humanized, is derived from a murine immunoglobulin, or is derived from an immunoglobulin from another species, including, for example a rat, a pig, a monkey, or a camelid.

According to certain embodiments of the present invention, the binding domain polypeptide comprises, consists essentially of, or consists of, (a) at least one native or engineered immunoglobulin light chain variable region polypeptide; (b) at least one native or engineered immunoglobulin heavy chain variable region polypeptide; and (c) at least one linker polypeptide that is fused or otherwise connected to the polypeptide of (a) and to the polypeptide of (b). In certain further embodiments the native or engineered immunoglobulin light chain variable region and heavy chain variable region polypeptides are derived or constructed from human immunoglobulins, and in certain other further embodiments the linker polypeptide comprises at least one polypeptide including or having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:____]. In other embodiments the linker polypeptide comprises at least two or three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:____]. In other embodiments the linker comprises a glycosylation site, which in certain further embodiments is an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site. In another embodiment at least one of a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide is derived from an IgG or IgA human immunoglobulin heavy chain. In another embodiment at least one of a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide and a

native or engineered immunoglobulin heavy chain CH4 constant region polypeptide is derived from an IgE human immunoglobulin heavy chain.

An immunoglobulin hinge region polypeptide may comprise, consist essentially or, or consist of, for example, any of (1) any hinge or hinge-acting peptide or polypeptide that occurs naturally for example, a human immunoglobulin hinge region polypeptide including, for example, a wild-type human IgG hinge or a portion thereof, a wild-type human IgA hinge or a portion thereof, a wild-type human IgD hinge or a portion thereof, or a wild-type human IgE hinge-acting region, *i.e.*, IgE CH2, or a portion thereof, a wild-type camelid hinge region or a portion thereof (including a IgG1 llama hinge region or portion thereof, a IgG2 llama hinge region or portion thereof, and a IgG3 llama hinge region or portion thereof), a nurse shark hinge region or portion thereof, and/or a spotted ratfish hinge region or a portion thereof; (2) a mutated or otherwise altered or engineered hinge region polypeptide that contains no cysteine residues and that is derived or constructed from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues; (3) a mutated or otherwise altered or engineered hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues; (4) a hinge region polypeptide that has been mutated or otherwise altered or engineered to contain or add one or more glycosylation sites, for example, an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site; (5) a mutated or otherwise altered or engineered hinge region polypeptide in which the number of cysteine residues is reduced by amino acid substitution or deletion, for example, a mutated or otherwise altered or engineered IgG1 or IgG4 hinge region containing for example zero, one, or two cysteine residues, a mutated or otherwise altered or engineered IgG2 hinge region containing for example zero, one, two or three cysteine residues, a mutated or otherwise altered or engineered IgG3 hinge region containing for example zero, one, two, three, or from four to ten cysteine residues, or a mutated or otherwise altered or engineered human IgA1 or IgA2 hinge region polypeptide that contains zero or only one or two cysteine residues (*e.g.*, an "SCC" hinge), a mutated or otherwise altered or engineered IgD hinge region containing no cysteine residues, or a mutated or otherwise altered or engineered

human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide that contains zero or only one, two, three or four cysteine residues; or (6) any other connecting region molecule described or referenced herein or otherwise known or later discovered as useful for connecting adjoining immunoglobulin domains such as, for example, a CH1 domain and a CH2 domain. For example, a hinge region polypeptide may be selected from the group consisting of (i) a wild-type human IgG1 immunoglobulin hinge region polypeptide, for example, (ii) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived or constructed from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 or other immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated, (iii) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 or other immunoglobulin hinge region polypeptide contains no more than one cysteine residue, and (iv) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide contains no cysteine residues. In certain embodiments, for example, the immunoglobulin hinge region polypeptide is a mutated or otherwise altered or engineered hinge region polypeptide and exhibits a reduced ability to dimerize, relative to a wild-type human immunoglobulin G or other wild type hinge region or hinge-acting polypeptide.

The immunoglobulin heavy chain constant region polypeptides may be, for example, native or engineered CH2 and CH3 domains of an isotype that is human IgG or human IgA. The immunoglobulin heavy chain constant region polypeptides may also be, for example, native or engineered immunoglobulin heavy chain constant region CH3 and CH4 polypeptides of an isotype that is human IgE.

In certain other embodiments the target or target antigen may be, for example, CD19 (B-lymphocyte antigen CD19, also referred to as B-lymphocyte surface

antigen B4, or Leu-12), CD20 (B-lymphocyte antigen 20, also referred to as B-lymphocyte surface antigen B1, Leu-16, or Bp35), CD22 (B-cell receptor CD22, also referred to as Leu-14, B-lymphocyte cell adhesion molecule, or BL-CAM), CD37 (leukocyte antigen CD37), CD40 (B-cell surface antigen CD40, also referred to as Tumor Necrosis Factor receptor superfamily member 5, CD40L receptor, or Bp50), CD80 (T lymphocyte activation antigen CD80, also referred to as Activation B7-1 antigen, B7, B7-1, or BB1), CD86 (T lymphocyte activation antigen CD86, also referred to as Activation B7-2 antigen, B70, FUN-1, or BU63), CD137 (also referred to as Tumor Necrosis Factor receptor superfamily member 9), CD152 (also referred to as cytotoxic T-lymphocyte protein 4 or CTLA-4), CD45 (Leukocyte common antigen, also referred to as L-CA, T200, and EC 3.1.3.48), CD45RA (an isoform of CD45, and an antigen expressed on naïve or immature lymphocytes), CD45RB (an isoform of CD45), CD45RO (an isoform of CD45, and a common leukocyte antigen expressed on memory B and T cells), L6 (Tumor-associated antigen L6, also referred to as Transmembrane 4 superfamily member 1, Membrane component surface marker 1, or M3S1), CD2 (T-cell surface antigen CD2, also referred to as T-cell surface antigen T11/Leu-5, LFA-2, LFA-3 receptor, Erythrocyte receptor, or Rosette receptor), CD28 (T-cell-specific homodimer surface protein CD28, also referred to as Tp44), CD30 (lymphocyte activation antigen CD30, also referred to as Tumor Necrosis Factor receptor superfamily member 8, CD30L receptor, or Ki-1), CD50 (also referred to as Intercellular adhesion molecule-3 (ICAM3), or ICAM-R), CD54 (also referred to as Intercellular adhesion molecule-1 (ICAM1), or Major group rhinovirus receptor), B7-H1 (ligand for an immunoinhibitory receptor expressed by activated T cells, B cells, and myeloid cells, also referred to as PD-L1; see Dong, *et al.*, "B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion," 1999 *Nat. Med.* 5:1365-1369), CD134 (also referred to as Tumor Necrosis Factor receptor superfamily member 4, OX40, OX40L receptor, ACT35 antigen, or TAX-transcriptionally activated glycoprotein 1 receptor), 41BB (4-1BB ligand receptor, T-cell antigen 4-1BB, or T-cell antigen ILA), CD153 (also referred to as Tumor Necrosis Factor ligand superfamily member 8, CD30 ligand, or CD30-L), CD154 (also referred to as Tumor Necrosis Factor ligand superfamily member 5, CD40 ligand, CD40-L, TNF-related activation protein, TRAP, or T cell antigen

Gp39), ICOS (Inducible Costimulator), CD3 (one or more of the delta, epsilon, gamma, eta and/or zeta chains, alone or in combination), CD4 (T-cell surface glycoprotein CD4, also referred to as T-cell surface antigen T4/Leu-3), CD25 (also referred to as Interleukin-2 receptor alpha chain, IL-2 receptor alpha subunit, p55, or Tac antigen), CD8 α (T-cell surface glycoprotein CD8 alpha chain, also referred to as T-lymphocyte differentiation antigen, T8/Leu-2, and Lyt-2), CD11b (also referred to as Integrin alpha-M, Cell surface glycoprotein MAC-1 alpha subunit, CR-3 alpha chain, Leukocyte adhesion receptor Mo1, or Neutrophil adherence receptor), CD14 (Monocyte differentiation antigen CD14, also referred to as Myeloid cell-specific leucine-rich glycoprotein or LPS receptor), CD56 (also referred to as Neural cell adhesion molecule 1), or CD69 (also referred to as Early T-cell activation antigen p60, Gp32/28, Leu-23, MLR-3, Activation inducer molecule, or AIM). The above list of construct targets and/or target antigens is exemplary only and is not exhaustive.

In another aspect, the invention includes a binding construct (or a polynucleotide encoding such a construct) that comprises a CD154 extracellular domain, or desired functional portion thereof. In one embodiment of this aspect of the invention, for example, the binding construct comprises a CD154 extracellular domain fused or otherwise connected to a second binding domain. The second binding domain, for example, may comprise, consist essentially of, or consist of at least one immunoglobulin variable region polypeptide. The at least one immunoglobulin variable region polypeptide may be a native or engineered scFv. The native or engineered scFv may be a native or engineered scFv disclosed or described herein. The second binding domain, including a native or engineered scFv, may be one that binds, for example, to any of the targets, including target antigens, disclosed or described herein, including but not limited to, for example, any of B7-H1, ICOS, L6, CD2, CD3, CD8, CD4, CD11b, CD14, CD19, CD20, CD22, CD25, CD28, CD30, CD37, CD40, CD45, CD50, CD54, CD56, CD69, CD80, CD86, CD134, CD137, CD152, CD153, or CD154.

In another embodiment the binding domain polypeptide comprises a CTLA-4 extracellular domain, or desired functional portion thereof, and in further embodiments at least one of the immunoglobulin heavy chain constant region polypeptides selected from a

CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgG1 constant region polypeptide, either native or engineered.

In another further embodiment at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a
5 CH3 constant region polypeptide is a human IgA constant region polypeptide, either native or engineered.

In another further embodiment at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH3 constant region polypeptide and a CH4 constant region polypeptide is a human IgE constant region polypeptide, either native
10 or engineered.

Turning to another embodiment, the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide; (b) a native or engineered immunoglobulin
15 heavy chain CH2 (or IgE Ch3) constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 (or IgE CH4) constant region polypeptide that is fused or otherwise connected to the CH2 (or IgE CH3) constant region polypeptide, wherein (1) the binding domain polypeptide comprises a CTLA-4 extracellular domain, or a portion thereof, that is
20 capable of binding or specifically binding to at least one CTLA-4 ligand selected from the group consisting of CD80 and CD86, (2) the immunoglobulin hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide that is selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG1 hinge region
25 polypeptide, and a native or engineered human IgE CH2 region polypeptide (3) a immunoglobulin heavy chain constant region polypeptide that comprises, consists essentially of, or consists of, a polypeptide that is selected from the group consisting of a native or engineered human IgA heavy chain CH2 constant region polypeptide, a native or engineered human IgG1 heavy chain CH2 constant region polypeptide, and a native or
30 engineered human IgE heavy chain CH3 constant region polypeptide (4) a immunoglobulin

heavy chain constant region polypeptide that comprises, consists essentially of, or consists of, a polypeptide that is selected from the group consisting of a native or engineered human IgA heavy chain CH3 constant region polypeptide, a native or engineered human IgG1 heavy chain CH3 constant region polypeptide, and a native or engineered human IgE heavy chain CH4 constant region polypeptide, and (5) the binding domain-immunoglobulin fusion protein is capable of inducing at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation.

In another embodiment the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a native or engineered human IgE hinge-acting region, *i.e.*, a IgE CH2 region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgE CH3 constant region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first native or engineered constant region polypeptide, wherein said native or engineered second constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgE CH4 constant region polypeptide and wherein (1) the binding domain-immunoglobulin fusion protein is capable of inducing at least one immunological activity selected from antibody dependent cell-mediated cytotoxicity and induction of an allergic response mechanism, and (2) the binding domain polypeptide is capable of binding or specifically binding to an antigen. In a further embodiment the antigen is a tumor antigen.

In certain other embodiments the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an

immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one antigen that is present on an immune effector cell and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide

5 selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG hinge region polypeptide, and a native or engineered human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said first native or

10 engineered constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, and a native or engineered human IgE CH3 constant region polypeptide; (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is

15 fused or otherwise connected to the first constant region polypeptide, wherein said second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, and a native or engineered human IgE CH4 constant region polypeptide; and (d) a native or

20 engineered plasma membrane anchor domain polypeptide. In one example of this embodiment, the plasma membrane anchor domain polypeptide links to a membrane via a native or engineered glycosyl-phosphatidylinositol-linkage. In a further embodiment the plasma membrane anchor domain polypeptide comprises, consists essentially of, or consists of, a native or engineered transmembrane domain polypeptide. In another further

25 embodiment the membrane anchor domain polypeptide comprises, consists essentially of, or consists of, a native or engineered transmembrane domain polypeptide and a native or engineered cytoplasmic tail polypeptide. In a still further embodiment the cytoplasmic tail polypeptide comprises, consists essentially of, or consists of, a native or engineered apoptosis signaling polypeptide sequence, which in a still further embodiment is derived or

30 constructed from a native or engineered receptor death domain polypeptide, a death domain,

or a functional portion of either. In a further embodiment the native or engineered death domain polypeptide comprises, consists essentially of, or consists of, for example, a native or engineered polypeptide selected from an ITIM domain (immunoreceptor Tyr-based inhibition motif), an ITAM domain (immunoreceptor Tyr-based activation motif), TRAF, RIP, CRADD, FADD (Fas-associated death domain), TRADD (Tumor Necrosis Factor receptor type 1 associated DEATH domain protein), RAIDD (also referred to as RAID), CD95 (Tumor Necrosis Factor receptor superfamily member 6, also referred to as FASL receptor, Apoptosis-mediating surface antigen FAS, FAS and Apo-1 antigen), TNFR1, and/or DR5 (death receptor-5). In another embodiment the native or engineered apoptosis signaling polypeptide sequence comprises, consists essentially of, or consists of, for example, a polypeptide sequence derived from a native or engineered caspase polypeptide that is caspase-3 or caspase-8 or caspase-10, including caspase 8/FLICE/MACH/Mch5 and caspase 10/Flice2/Mch4. In another embodiment the plasma membrane anchor domain polypeptide comprises, consists essentially of, or consists of, for example, a native or engineered glycosyl-phosphatidylinositol-linkage polypeptide sequence. In another embodiment the antigen that is present on an immune effector cell is, for example, CD2, CD16, CD28, CD30, CD32, CD40, CD50, CD54, CD64, CD80, CD86, B7-H1, CD134, CD137, CD152, CD153, CD154, ICOS, CD19, CD20, CD22, CD37, L6, CD3, CD4, CD25, CD8, CD11b, CD14, CD56, or CD69. In another embodiment the human IgG is a native or engineered human IgG1. These binding domain-immunoglobulin fusion proteins may be capable of inducing, for example, at least one immunological activity selected from antibody dependent cell-mediated cytotoxicity and/or complement fixation and/or CDC, and are capable of binding or specifically binding to a target, including, for example, a target antigen. Immune effector cells include, for example, granulocytes, mast cells, monocytes, macrophages, dendritic cells, neutrophils, eosinophils, basophils, NK cells, T cells (including Th1 cells, Th2 cells, Tc cells, memory T cells, null cells, and large granular lymphocytes, *etc.*), and B cells. This embodiment of the invention further includes the use of such proteins for therapy, and, for example, the use of such vectors for *in vivo* and *ex vivo* gene therapy. The above lists of construct components and targets are not exhaustive and

may include any desired target or component that may function as, or be useful for the purposes, described herein.

In another embodiment, the invention provides a protein having a first protein motif that comprises, consists essentially of, or consists of, (1) a native or engineered immunoglobulin hinge region or hinge-acting region (*e.g.*, IgE CH2) polypeptide that is fused or otherwise connected to (2) a native or engineered CH2 constant region polypeptide (or native or engineered IgE CH3 constant region polypeptide). Said first protein motif may be fused or otherwise connected to one or more other such first protein motifs to form a second protein motif, the second protein motif being fused or otherwise connected to (3) a native or engineered CH3 constant region (or a native or engineered IgE CH4 constant region) to form a third protein motif. Said first, second or third protein motifs may be fused or otherwise connected to one or more of the herein-described native or engineered plasma membrane anchor domain polypeptides, including, for example, a native or engineered transmembrane domain polypeptide, and a native or engineered transmembrane domain polypeptide and a native or engineered cytoplasmic tail polypeptide, such as for example, a native or engineered apoptosis signaling polypeptide sequence, which may be derived or constructed from a native or engineered receptor death domain polypeptide, a death domain, or a functional portion of either. Thus, a protein or polynucleotide within this aspect of the invention may be, for example, a Hinge-CH2-CH3-TransmembraneDomain-DeathDomain construct. It may also be, for example, a (Hinge-CH2)_X-CH3- TransmembraneDomain-DeathDomain construct, where X is from 2 to about 5, or such other number as may be needed to achieve a desired length or Fc receptor binding and/or complement fixation function(s). This embodiment of the invention also includes polynucleotides encoding such proteins, vectors including such polynucleotides, and host cells containing such polynucleotides and vectors. This embodiment of the invention further includes the use of such proteins for therapy, and, for example, the use of such polynucleotides and/or vectors for *in vivo* and *ex vivo* gene therapy. The invention provides, in another embodiment, a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is

capable of binding or specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a
5 native or engineered human IgG hinge region polypeptide, and a native or engineered human IgE hinge-acting region, *i.e.*, IgE CH2, region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein the first constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or
10 engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, or a native or engineered human IgE CH3 constant region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein the second constant region polypeptide comprises, consists essentially
15 of, or consists of, a polypeptide that is a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, or a native or engineered human IgE CH4 constant region polypeptide. In a further embodiment the human IgG polypeptides are native or engineered human IgG1 polypeptides.

In another embodiment the present invention provides a binding domain-
20 immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide may be as described above or herein, and may comprises, consist essentially of, or consist of, for example, a wild-type or engineered human IgA hinge region polypeptide; (b) a native or engineered
25 immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered CH2 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgA CH2 constant region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise
30 connected to the native or engineered CH2 constant region polypeptide, wherein the native

or engineered CH3 constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is (i) a wild-type human IgA CH3 constant region polypeptide or other IgA region, preferably human or humanized, that is capable of associating with J Chain, (ii) a mutated, altered or otherwise engineered human IgA CH3 constant region polypeptide that is, for example, incapable of associating with a J chain, wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target such as, for example, an antigen. In certain further embodiments the mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain is (i) a polypeptide comprising, consisting essentially of, or consisting of, an amino acid sequence as set forth in SEQ ID NO:___ or (ii) a polypeptide comprising, consisting essentially of, or consisting of, an amino acid sequence as set forth in SEQ ID NO:___ . In other embodiments, the IgA hinge region polypeptide is a native or engineered IgA1 hinge region polypeptide or a native or engineered IgA2 hinge region polypeptide. In still other embodiments, the IgA hinge region polypeptide is different from a wild-type IgA1 or IgA2 hinge region polypeptide by, for example, the alteration, substitution, or deletion of one or more of the cysteine residues within said wild-type hinge region.

In certain other embodiments the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide; (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein the native or engineered CH2 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered llama CH2 constant region polypeptide that is a native or engineered llama IgG1 CH2 constant region polypeptide, a native or engineered llama IgG2 CH2 constant region polypeptide, or a native or engineered llama IgG3 CH2 constant region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise

connected to the native or engineered CH2 constant region polypeptide, wherein said native or engineered CH3 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered llama CH3 constant region polypeptide that is selected from the group consisting of a native or engineered llama IgG1 CH3 constant region polypeptide, a native or engineered llama IgG2 CH3 constant region polypeptide and a native or engineered llama IgG3 CH3 constant region polypeptide wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, fixation of complement and CDC, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target, for example a target antigen. In a further embodiment the immunoglobulin hinge region polypeptide, the native or engineered llama CH2 constant region polypeptide and the native or engineered llama CH3 constant region polypeptide comprise sequences derived from a native or engineered llama IgG1 polypeptide and the fusion protein does not include a native or engineered llama IgG1 CH1 domain. In certain embodiments the invention provides any of the above described binding domain-immunoglobulin fusion proteins wherein the hinge region polypeptide is mutated, engineered, or otherwise altered to contain a glycosylation site, which in certain further embodiments is an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site.

In certain embodiments the invention, there are provided any of the above or herein described binding constructs, including binding domain-immunoglobulin fusion proteins, wherein a binding region or binding domain polypeptide comprises two or more binding domain polypeptide sequences wherein each of the binding domain polypeptide sequences is capable of binding or specifically binding to a target(s) such as an antigen(s), which target(s) or antigen(s) may be the same or may be different. A native, for more preferably an engineered, IgD hinge is a desired connecting region between binding domains of a bispecific molecule of the invention, *i.e.*, one with two or more binding domains, preferably two. The wild type human IgD hinge has one cysteine that forms a disulfide bond with the light chain in the native IgD structure. It is desirable to mutate or delete this cysteine in the human IgD hinge for use as a connecting region between binding domains of,

for example, a bispecific molecule. Other amino acid changes or deletions or alterations in an IgD hinge that do not result in undesired hinge inflexibility are within the scope of the invention. native or engineered IgD hinge regions from other species are also within the scope of the invention, as are humanized native or engineered IgD hinges from non-human species. The present invention also provides, in certain embodiments, a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, an alternative hinge region polypeptide sequence; (b) a first native or engineered immunoglobulin heavy chain constant region, such as an IgG or IgA CH2 constant region polypeptide (or an IgE CH3 constant region polypeptide) that is fused or otherwise connected to the hinge region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region, such as an IgG or IgA CH3 constant region polypeptide (or an IgE CH4 constant region polypeptide) that is fused or otherwise connected to the first constant region polypeptide, wherein: (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target, such as an antigen.

Turning to another embodiment there is provided a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one target, such as an antigen, that is present on a cancer cell surface and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, an alternative hinge region polypeptide sequence; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered constant region polypeptide comprises, consists

essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, and a native or engineered human IgE CH3 constant region polypeptide; and (c) a second immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein the second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, or a native or engineered human IgE CH4 constant region polypeptide. In certain further embodiments the alternative hinge region polypeptide sequence comprises, consists essentially of, or consists of, a polypeptide sequence of at least ten continuous amino acids that are present in a sequence selected from SEQ ID NOS: __-__.

In certain embodiments the present invention provides polynucleotides or vectors (including cloning vectors and expression vectors) or transformed or transfected cells, including isolated or purified or pure polynucleotides, vectors, and isolated transformed or transfected cells, encoding or containing any one of the above or herein described polypeptide or protein constructs of the invention, for example, including binding domain-immunoglobulin fusion proteins. Thus, in various embodiments the invention provides a recombinant cloning or expression construct comprising any such polynucleotide that is operably linked to a promoter.

In other embodiments there is provided a host cell transformed or transfected with, or otherwise containing, any such recombinant cloning or expression construct. Host cells include the cells of a subject undergoing *ex vivo* cell therapy including, for example, *ex vivo* gene therapy.

In a related embodiment there is provided a method of producing a polypeptide or protein or other construct of the invention, for example, including a binding domain-immunoglobulin fusion protein, comprising the steps of (a) culturing a host cell as described or provided for herein under conditions that permit expression of the construct, for example, a binding domain-immunoglobulin fusion protein; and (b) isolating the construct,

for example, the binding domain-immunoglobulin fusion protein from the host cell or host cell culture.

In another embodiment there is provided a pharmaceutical composition comprising any one of the above or herein described polypeptide or protein or other
5 constructs of the invention, for example (including, for example, binding domain-immunoglobulin fusion proteins), in combination with a physiologically acceptable carrier.

In another embodiment the invention provides a pharmaceutical composition comprising, for example, an isolated, purified, or pure polynucleotide encoding any one of the polypeptide or protein constructs of the invention, for example (including, for example,
10 binding domain-immunoglobulin fusion proteins), in combination with a physiologically acceptable carrier, or for example, in combination with, or in, a gene therapy delivery vehicle or vector.

In another embodiment the invention provides a method of treating a subject having or suspected of having a malignant condition or a B cell disorder, comprising
15 administering to a patient a therapeutically effective amount of any of the pharmaceutical compositions described or claimed herein.

In certain further embodiments the malignant condition or B cell disorder is a B cell lymphoma or B cell leukemia, or a disease characterized by autoantibody production, and in certain other further embodiments the B cell disorder is, for example, rheumatoid
20 arthritis, myasthenia gravis, Grave's disease, type I diabetes mellitus, multiple sclerosis or an autoimmune disease. In certain other embodiments the malignant condition is, for example, melanoma, myeloma, glioma, astrocytoma, lymphoma, leukemia, carcinoma, or sarcoma, and so on.

It is another aspect of the present invention to provide a binding domain-immunoglobulin fusion protein, comprising, consisting essentially or, or consisting of, (a) a
25 binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide is as described herein, and may be selected from the group consisting of (i) a mutated, engineered or otherwise altered hinge region polypeptide that contains no cysteine residues and that is derived from a wild-
30 type immunoglobulin hinge region polypeptide having one or more cysteine residues, (ii) a

mutated, engineered or otherwise altered hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type immunoglobulin hinge region polypeptide having two or more cysteine residues, (iii) a wild-type human IgA hinge region polypeptide, (iv) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains no cysteine residues, (v) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains one cysteine residue and (vi) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains two cysteine residues; (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise connected to the CH2 constant region polypeptide, wherein: (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to an antigen. In one embodiment the immunoglobulin hinge region polypeptide is a mutated hinge region polypeptide, for example, and the resulting construct exhibits a reduced ability to dimerize, relative to a construct containing a wild-type human immunoglobulin G hinge region polypeptide. In another embodiment the binding domain polypeptide comprises, consists essentially of, or consists of, at least one native or engineered immunoglobulin variable region polypeptide that is a native or engineered immunoglobulin light chain variable region polypeptide and/or a native or engineered immunoglobulin heavy chain variable region polypeptide. In a further embodiment the native or engineered immunoglobulin variable region polypeptide is derived from a human immunoglobulin and, for example, may be humanized.

In another embodiment, the invention provides a binding domain-immunoglobulin fusion protein includes a binding domain polypeptide that comprises, consists essentially of, or consists of, (a) at least one native or engineered immunoglobulin light chain variable region polypeptide; (b) at least one native or engineered immunoglobulin heavy chain variable region polypeptide; and (c) at least one linker peptide that is fused or otherwise connected to the polypeptide of (a) and to the polypeptide of (b).

In a further embodiment the native or engineered immunoglobulin light chain variable region and the native or engineered heavy chain variable region polypeptides are derived from human immunoglobulins and may, for example, be humanized.

In another embodiment at least one of the native or engineered immunoglobulin heavy chain CH2 (or IgE CH3) constant region polypeptide and the native or engineered immunoglobulin heavy chain CH3 (or IgE CH4) constant region polypeptide is derived or constructed from a human immunoglobulin heavy chain. In another embodiment the native or engineered immunoglobulin heavy chain constant region CH2 and CH3 polypeptides are of, or are derived or otherwise prepared or constructed from, an isotype selected from human IgG and human IgA. In another embodiment the target, for example, the target antigen is selected from the group consisting of CD16, CD19, CD20, CD37, CD40, CD45RO, CD80, CD86, CD137, CD152, and L6. In certain further embodiments of the above described fusion protein construct, the binding domain comprises, consists essentially of, or consists of, an scFv and the scFv contains a linker polypeptide that comprises, consists essentially of, or consists of, at least one polypeptide comprising or having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:___], and in certain other embodiments the linker polypeptide comprises, consists essentially of, or consists of, at least three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:___]. In certain embodiments the immunoglobulin hinge region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgG, IgA, IgD hinge region polypeptide, or a native or engineered IgE CH2 region polypeptide. In certain embodiments the binding domain polypeptide comprises, consists essentially of, or consists of, a native or engineered CD154 extracellular domain. In certain embodiments the binding domain polypeptide comprises, consists essentially of, or consists of, a native or engineered CD154 extracellular domain and at least one a native or engineered immunoglobulin variable region polypeptide.

In other embodiments the invention provides an isolated polynucleotide encoding any of the constructs of the invention, for example, protein or polypeptide constructs of the invention including binding domain-immunoglobulin fusion proteins, and in related embodiments the invention provides a recombinant expression construct

comprising such a polynucleotide, and in certain further embodiments the invention provides a host cell transformed or transfected with, or otherwise containing, such a recombinant expression construct. In another embodiment the invention provides a method of producing a construct of the invention, for example, a protein or polypeptide construct of the invention such as a binding domain-immunoglobulin fusion protein, comprising the steps of (a) culturing a host cell that has been transformed or transfected with, or otherwise made to contain, a polynucleotide construct of the invention under conditions that permit expression of the construct, for example, a construct encoding a binding domain-immunoglobulin fusion protein; and (b) isolating the construct, for example, the binding domain-immunoglobulin fusion protein, from the host cell culture.

The inventions described and claimed herein include novel molecules useful, for example, as therapeutics and other purposes including diagnostic and research purposes. Such molecules have, for example, antigen-binding or other binding function(s) and one or more effector functions. DNA constructs of the invention are useful in, for example, gene therapies, including *in vivo* and *ex vivo* gene therapies.

In one aspect, various constructs of the molecules of the invention include molecules comprising a “binding region”, a “tail” region, and a “connecting” region that joins a binding region and a tail region.

Binding regions within the molecules of the invention may comprise, for example, binding domains for desired targets, including antigen-binding targets. Binding domains for antigen binding targets may comprise, for example, single chain Fvs and scFv domains. In certain embodiments, molecules of the invention may comprise a binding region having at least one immunoglobulin variable region polypeptide, which may be a light chain or a heavy chain variable region polypeptide. In certain embodiments, molecules of the invention may comprise at least one such light chain V-region and one such heavy chain V-region and at least one linker peptide that connects the V-regions. ScFvs useful in the invention also include those with chimeric binding or other domains or sequences. Other ScFvs useful in the invention also include those with humanized binding or other domains or sequences. In such embodiments, all or a portion of an immunoglobulin binding or other sequence that is derived from a non-human source may be “humanized” according to

recognized procedures for generating humanized antibodies, *i.e.*, immunoglobulin sequences into which human Ig sequences are introduced to reduce the degree to which a human immune system would perceive such proteins as foreign.

Example of scFvs useful in the invention, whether included as murine or
5 other scFvs (including human scFvs), chimeric scFvs, or humanized scFvs, in whole or in part, include anti-human CD20 scFvs (for example, "2H7" scFvs), anti-human CD37 scFvs (for example, "G28-1" scFvs), anti-human CD40 scFvs (for example, "G28-5" scFvs and "40.2.220" scFvs), anti-carcinoma-associated antigen scFvs (for example, "L6" scFvs), anti-CTLA-4 (CD152) scFvs (for example, "10A8" scFvs), anti-human CD28 scFvs (for
10 example, "2E12" scFvs), anti-murine CD3 scFvs (for example, "500A2" scFvs), anti-human CD3 scFvs (for example, G19-4 scFvs), anti-murine 4-1BB scFvs (for example, "1D8" scFvs), anti-human 4-1BB scFvs (for example, "5B9" scFvs), anti-human CD45RO (for example, "UCHL-1" scFvs), and anti-human CD16 (for example, "Fc2" scFvs).

scFvs useful in the invention also include scFvs, including chimeric and
15 humanized scFvs, having one or more amino acid substitutions. A preferred amino acid substitution is at amino acid position 11 in the variable heavy chain (the V_H). Such a substitution may be referred to herein as "Xxx V_H 11Zxx". Thus, for example, where the normally occurring amino acid at position V_H 11 is a Leucine, and a Serine amino acid residue is substituted therefor, the substitution is identified as "L V_H 11S" or "Leu V_H 11Ser."
20 Other preferred embodiments of the invention include molecules containing scFvs wherein the amino acid residue normally found at position V_H 11 is deleted. Still other preferred embodiments of the invention include molecules containing scFvs wherein the amino acid residues normally found at positions V_H 10 and/or V_H 11 and/or V_H 12 are substituted or deleted.

25 Other binding regions within the molecules of the invention may include domains that comprise sites for glycosylation, for example, covalent attachment of carbohydrate moieties such as monosaccharides or oligosaccharides.

Still other binding regions within molecules of the invention include polypeptides that may comprise proteins or portions thereof that retain the ability to
30 specifically bind another molecule, including an antigen. Thus, binding regions may

comprise or be derived from hormones, cytokines, chemokines, and the like; cell surface or soluble receptors for such polypeptide ligands; lectins; intercellular adhesion receptors such as specific leukocyte integrins, selectins, immunoglobulin gene superfamily members, intercellular adhesion molecules (ICAM-1, -2, -3) and the like; histocompatibility antigens; and so on. Binding regions derived from such molecules generally will include those portions of the molecules necessary or desired for binding to a target.

Certain constructs include binding regions that comprise receptor or receptor-binding domains. Receptor domains useful for binding to a target include, for example, a CD154 extracellular domain, or a CTLA-4 extracellular domain. In another example, the binding domain may include a first portion comprising, consisting essentially or, or consisting of, a CD154 extracellular domain and a second portion comprising, consisting essentially or, or consisting of, at least one immunoglobulin variable region polypeptide, said second portion including, for example, an scFv or a V_H. Examples of other cell surface receptors that may comprise, consist essentially or, or consist of, or a portion of which may provide, a binding region or binding domain polypeptide, include, for example, HER1, HER2, HER3, HER4, epidermal growth factor receptor (EGFR), vascular endothelial cell growth factor, vascular endothelial cell growth factor receptor, insulin-like growth factor-I, insulin-like growth factor-II, transferrin receptor, estrogen receptor, progesterone receptor, follicle stimulating hormone receptor (FSH-R), retinoic acid receptor, MUC-1, NY-ESO-1, Melan-A/MART-1, tyrosinase, Gp-100, MAGE, BAGE, GAGE, any of the CTA class of receptors including in particular HOM-MEL-40 antigen encoded by the SSX2 gene, carcinoembryonic antigen (CEA), and PyLT. Additional cell surface receptors that may be sources of binding regions or binding domain polypeptides include, for example, CD2, 4-1BB, 4-1BB ligand, CD5, CD10, CD27, CD28, CD152/CTLA-4, CD40, interferon- γ (IFN- γ), interleukin-4 (IL-4), interleukin-17 (IL-17) and interleukin-17 receptor (IL-17R). Still other cell surface receptors that may be sources of binding regions and/or binding domain polypeptides include, for example, CD59, CD48, CD58/LFA-3, CD72, CD70, CD80/B7.1, CD86/B7.2, B7-H1/B7-DC, IL-17, CD43, ICOS, CD3 (*e.g.*, gamma subunit, epsilon subunit, delta subunit), CD4, CD25, CD8, CD11b, CD14, CD56, CD69 and VLA-4 ($\alpha_4\beta_7$). The following cell surface receptors are typically associated with B cells: CD19, CD20,

CD22, CD30, CD153 (CD30 ligand), CD37, CD50 (ICAM-3), CD106 (VCAM-1), CD54 (ICAM-1), interleukin-12, CD134 (OX40), CD137 (41BB), CD83, and DEC-205. These lists are not exhaustive. Binding regions such as those set forth above may be connected, for example, by a native or engineered IgD hinge region polypeptide, preferably a human or humanized native or engineered IgD hinge region polypeptide. The invention thus further provides constructs that comprise, consist essentially of, or consist of, two binding regions, for example, an scFv and a cell surface receptor (or portion thereof), connected by a third molecule, for example, an IgD hinge region polypeptide as described herein.

Various molecules of the invention described and claimed herein include a connecting region joining one end of the molecule to another end. Such connecting regions may comprise, for example, immunoglobulin hinge region polypeptides, including any hinge peptide or polypeptide that occurs naturally. A connecting region may also include, for example, any artificial peptide or other molecule (including, for example, non-peptide molecules, partial peptide molecules, and peptidomimetics, *etc.*) useful for joining the tail region and the binding region. These may include, for example, alterations of molecules situated in an immunoglobulin heavy chain polypeptide between the amino acid residues responsible for forming intrachain immunoglobulin-domain disulfide bonds in CH1 and CH2 regions. Naturally occurring hinge regions include those located between the constant region domains, CH1 and CH2, of an immunoglobulin. Useful immunoglobulin hinge region polypeptides include, for example, human immunoglobulin hinge region polypeptides and llama or other camelid immunoglobulin hinge region polypeptides. Other useful immunoglobulin hinge region polypeptides include, for example, nurse shark and spotted ratfish immunoglobulin hinge region polypeptides. Human immunoglobulin hinge region polypeptides include, for example, wild type IgG hinges including wild-type human IgG1 hinges, human IgG-derived immunoglobulin hinge region polypeptides, a portion of a human IgG hinge or IgG-derived immunoglobulin hinge region, wild-type human IgA hinge region polypeptides, human IgA-derived immunoglobulin hinge region polypeptides, a portion of a human IgA hinge region polypeptide or IgA-derived immunoglobulin hinge region polypeptide, wild-type human IgD hinge region polypeptides, human Ig-D derived immunoglobulin hinge region polypeptides, a portion of a human IgD hinge region

polypeptide or IgD-derived immunoglobulin hinge region polypeptide, wild-type human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptides (which generally have 5 cysteine residues), human IgE-derived immunoglobulin hinge region polypeptides, a portion of a human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide or IgE-derived immunoglobulin hinge region polypeptide, and so on. A polypeptide “derived from” or that is “a portion or fragment of” an immunoglobulin polypeptide chain region regarded as having hinge function has one or more amino acids in peptide linkage, for example 15-115 amino acids, preferably 95-110, 80-94, 60-80, or 5-65 amino acids, preferably 10-50, more preferably 15-35, still more preferably 18-32, still more preferably 20-30, still more preferably 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acids. Llama immunoglobulin hinge region polypeptides include, for example, an IgG1 llama hinge. The connecting region may comprise a stretch of consecutive amino acids from an immunoglobulin hinge region. For example, the connecting region can comprise at least five consecutive hinge region amino acids, at least ten consecutive hinge region amino acids, at least fifteen consecutive hinge region amino acids, at least 20 consecutive hinge region amino acids, and at least twenty five or more consecutive hinge region amino acids from human IgG hinge, human IgA hinge, human IgE hinge, camelid hinge region, IgG1 llama hinge region, nurse shark hinge region, and spotted ratfish hinge region, including for example an IgG₁ hinge region, a IgG₂ hinge region, a IgG₃ hinge region, an IgG₃ hinge region, and an IgG₄ hinge region.

Such connecting regions also include, for example, mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides. A mutated or otherwise altered or engineered immunoglobulin hinge region polypeptide may comprise, consist essentially of, or consist of, a hinge region that has its origin in an immunoglobulin of a species, of an immunoglobulin isotype or class, or of an immunoglobulin subclass that is the same or different from that of any included native or engineered CH2 and CH3 domains. Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, a wild-type immunoglobulin hinge region that contains one or more cysteine residues, for example, a wild-type human IgG or IgA hinge region that naturally comprises three cysteines. In such polypeptides the number of cysteine residues may be reduced by amino acid substitution or deletion or truncation, for

example. These polypeptides include, for example, mutated human or other IgG1 or IgG4 hinge region polypeptides containing zero, one, or two cysteine residues, and mutated human or other IgA1 or IgA2 hinge region polypeptides that contain zero, one, or two cysteine residues. Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, a wild-type immunoglobulin hinge region that contains three or more cysteine residues, for example, a wild-type human IgG2 hinge region (which has 4 cysteines) or IgG4 hinge region (which has 11 cysteines). Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, an IgE CH2 wild-type immunoglobulin region that generally contains five cysteine residues. In such polypeptides the number of cysteine residues may be reduced by one or more cysteine residues by amino acid substitution or deletion or truncation, for example. Also included are an altered hinge region polypeptides in which cysteine residues in the hinge region are substituted with serine or one or more other amino acids that are less polar, less hydrophobic, more hydrophilic, and/or neutral. Such mutated immunoglobulin hinge region polypeptides include, for example, mutated hinge region polypeptides that contain one cysteine residue and that are derived from a wild-type immunoglobulin hinge region polypeptide having two or more cysteine residues, such as a mutated human IgG or IgA hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type human IgG or IgA region polypeptide. Connecting region polypeptides include immunoglobulin hinge region polypeptides that are compromised in their ability to form interchain, homodimeric disulfide bonds.

Mutated immunoglobulin hinge region polypeptides also include mutated hinge region polypeptides that exhibit a reduced ability to dimerize, relative to a wild-type human immunoglobulin G hinge region polypeptide, and mutated hinge region polypeptides that allow expression of a mixture of monomeric and dimeric molecules. Mutated immunoglobulin hinge region polypeptides also include hinge region polypeptides engineered to contain a glycosylation site. Glycosylation sites include, for example, an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site, and a phosphoglycation site.

Specific connecting regions useful in molecules of the invention described and claimed herein include, for example, the following 18 amino acid sequences, DQEPKSCDKTHTCPPCPA, DQEPKSSDKTHTSPPPSPA, and DLEPKSCDKTHTCPPCPA. Other specific connecting regions include, for example, the mutant hinges within the sequences referred to herein as “2H7 scFv (SSS-S)H WCH2 WCH3” and “2H7 scFv (CSS)H WCH2 WCH3”, and the human IgA-derived hinge referred to herein as “2H7 scFv IgAH WCH2 WCH3”.

Tail regions within the molecules of the invention may include heavy chain constant region immunoglobulin sequences. Tail regions may thus include, for example, a polypeptide having at least one of an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide. At least one of the immunoglobulin heavy chain CH2 constant region polypeptide and the immunoglobulin heavy chain CH3 constant region polypeptide may be derived from a human immunoglobulin heavy chain. Thus, for example, CH2 and/or CH3 polypeptides may be derived from human IgG, human IgA, or human IgD molecules. Tail regions may also include, for example, a polypeptide having at least one of an immunoglobulin heavy chain CH3 constant region polypeptide and an immunoglobulin heavy chain CH4 constant region polypeptide. At least one of the immunoglobulin heavy chain CH3 constant region polypeptide and the immunoglobulin heavy chain CH4 constant region polypeptide may be derived from a human immunoglobulin heavy chain. Thus, for example, CH3 and/or CH4 polypeptides may be derived from human IgE. An immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. An immunoglobulin heavy chain CH3 region polypeptide included within a molecule of the invention may also, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. Additionally, both the immunoglobulin heavy chain CH2 region polypeptide and the immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. In other molecules of the invention at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgA constant region

polypeptide. An immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgA1 and/or IgA2 subclasses. An immunoglobulin heavy chain CH3 region polypeptide included within a molecule of the invention may also, for example, be from the IgA1 and/or IgA2 subclasses. Additionally,

5 both the immunoglobulin heavy chain CH2 region polypeptide and the immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgA1 and/or IgA2 subclasses. In still other molecules of the invention, the tail region may comprise or consist essentially of a CH2 and/or CH3 constant region polypeptide comprising a polypeptide from human IgA and/or human IgE. In other

10 embodiments, for example, the tail region within a molecule of the invention may include an immunoglobulin heavy chain CH2 and/or CH3 constant region polypeptide that is a mutated (for example, a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain in which, for example, the IgA CH3 constant region polypeptide is of human origin). The tail region may also comprise, consist essentially of, or consist of an

15 extracellular portion of a protein from the TNF superfamily, for example, CD154.

For molecules of the invention intended for use in humans, these regions will typically be substantially or completely human to minimize a potential human immune responses against the molecules and to provide appropriate effector functions. In certain embodiments of the invention, for example, the tail region includes a human IgG1 CH3

20 region sequence, a wild-type IgA heavy chain constant region polypeptide sequence that is capable or incapable of associating with J chain.

In preferred embodiments of the invention, a CH1 domain is not included in the tail region of the molecule, and the carboxyl end of the binding region is joined to the amino terminus of a CH2 portion of a tail region either directly or indirectly. A binding

25 region may be indirectly joined to a tail region, for example via a connecting region polypeptide or other connecting molecule.

The invention also includes molecules that have mutated CH2 and/or CH3 sequences within a tail region. For example, a molecule of the invention may include a mutated Fc domain that has one or more mutations introduced into the CH2, CH3 and/or

30 CH4 domains. In certain embodiments of the invention, molecules may include an IgA CH3

constant region polypeptide such as a human IgA CH3 constant region polypeptide in which two or more residues from the C-terminus have been deleted to yield a truncated CH3 constant region polypeptide. In other embodiments of the invention, molecules include a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain that comprises a C-terminal deletion of either four or 18 amino acids. However, the invention need not be so limited, such that molecules containing the mutated IgA CH3 constant region polypeptide may comprise a deletion of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30 or more amino acids, so long as the fusion protein is capable of specifically binding an antigen and capable of at least one immunological activity such as ADCC, CDC or complement fixation. The invention also includes molecules containing a tail region that comprises a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain by virtue of replacement of the penultimate cysteine, or by chemical modification of that amino acid residue, in a manner that prevents interchain disulfide bond formation.

Various molecules of the invention include, for example, a binding domain scFv- fusion protein having a binding domain polypeptide comprising, consisting essentially of, or consisting of, (a) at least one immunoglobulin light chain variable region polypeptide, (b) at least one immunoglobulin heavy chain variable region polypeptide, and at least one linker peptide that joins the polypeptide of (a) and the polypeptide of (b). Such polypeptides may, for example, be derived from human immunoglobulins or non-human immunoglobulins.

Thus, in one aspect, the invention includes a non-naturally occurring single chain protein and/or V_H protein and/or V_L protein, or a desired portion of any of the above, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a flexible or other desired linker attached to said first polypeptide, a third polypeptide comprising a tail region, for example, an N-terminally truncated immunoglobulin heavy chain constant region polypeptide (or desired portion thereof) attached to the second polypeptide. The flexible linker may comprise, consist essentially of, or consist of, an immunoglobulin hinge region or portion thereof that has been mutated or otherwise altered or engineered, for example, one that

contains a number of cysteine residues that is less than the number of cysteine residues present in the wild type immunoglobulin hinge region or portion (for example, zero, one, or two cysteines in the case of IgG1 or IgG4), and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity, for example, ADCC, CDC, and/or complement fixation. This protein may include a binding domain polypeptide which is a single chain Fv. Additionally, this protein may include a binding domain polypeptide which is a single chain Fv wherein the heavy chain variable region of the single chain Fv has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112. The protein may also include a binding domain polypeptide which is a single chain Fv wherein the light chain variable region of the single chain Fv has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107.

In another aspect, the invention includes a non-naturally occurring V_H protein, or a desired portion thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule or construct, a V_H region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112 of said V_H region. Amino acids may be substituted with either naturally-occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of V_H proteins, or desired portions thereof, that comprise, consist essentially of, or consist of, alone or in combination with any other molecule or construct, a V_H region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112 of said V_H region. Such uses include uses in phage display, yeast display, and ribosome display systems and methods.

In yet another aspect, the invention includes a non-naturally occurring V_L protein, or a desired portion thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule, a V_L region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 of said V_L region. Amino acids may be substituted with either naturally-occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of V_L proteins, or desired portions thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule, a V_L region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 of said
5 V_L region. Such uses include uses in phage display, yeast display, and ribosome display systems and methods.

In yet another aspect, the invention includes a molecule comprising, consisting essentially of, or consisting of, (1) a V_H protein, or a desired portion thereof, wherein the V_H protein or portion thereof has an amino acid deletion or substitution at one or
10 more of amino acid positions 9, 10, 11, 12, 108, 110, and 112, and (2) a non-naturally occurring V_L protein, or a desired portion thereof, alone or in combination with any other molecule, wherein the V_L protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107. Amino acids may be substituted with either naturally-occurring or non-naturally occurring
15 amino acids, or any other desired useful molecule.

Also described and claimed are uses of a molecule comprising, consisting essentially of, or consisting of, (1) a V_H protein, or a desired portion thereof, wherein the V_H protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112, and (2) a non-naturally occurring V_L protein,
20 or a desired portion thereof, alone or in combination with any other molecule, wherein the V_L protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107. Such uses include uses in phage display, yeast display, and ribosome display systems and methods.

The invention also includes molecular constructs wherein the binding domain
25 is a single chain Fv and the heavy chain variable region of said single chain Fv has an amino acid substitution at amino acid position 11. The amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region may be selected from the group consisting of serine, threonine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine. The invention thus includes, for example, a construct
30 wherein the binding domain is a single chain Fv and the heavy chain variable region of said

single chain Fv has a serine amino acid substitution at amino acid position 11. Other amino acid position changes, substitutions, and deletions, are noted herein.

The invention also includes, for example, a construct wherein the binding domain is a single chain Fv and the amino acid at position 10 and/or 11 of the heavy chain
5 variable region of said single chain Fv has been deleted.

In another aspect, the invention includes constructs wherein the binding region binds to a tumor or tumor-associated antigen. The binding region of a construct of the invention may bind, for example, to a cancer cell antigen. Cancer cell antigens to which constructs of the invention bind include cancer cell surface antigens and intracellular cancer
10 cell antigens.

In yet another aspect, the invention includes a construct wherein the binding region binds to an antigen on an immune effector cell.

In another aspect, the invention includes a construct wherein the binding region binds to a B cell antigen including, for example, a B cell antigen selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86. Constructs of the
15 invention that bind to such B cell antigens include, for example, binding regions comprising an single chain Fv. Examples of such single chain Fv binding regions include molecules comprising or consisting essentially of single chain Fvs selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single
20 chain Fv. Other examples include a binding region comprising, consisting essentially of, or consisting of, an extracellular domain of CTLA-4.

In another aspect, the invention includes a construct wherein the binding region binds to a B cell differentiation antigen. B cell differentiation antigens include, for example, CD19, CD20, CD21, CD22, CD23, CD37, CD40, CD45RO, CD80, CD86, and
25 HLA class II.

In another aspect, the invention includes a construct wherein the binding region binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86,

CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

5 The invention also includes protein constructs having a binding region, a tail region, and a connecting region, wherein the protein construct is capable of existing in solution as a monomer or in substantially monomeric form.

 The invention also includes protein constructs having a binding region, a tail region, and a connecting region, wherein the protein construct is capable of forming a complex comprising two or more of said protein constructs including, for example, wherein said complex is a dimer.

10 In another aspect, constructs of the invention are capable of participating in or inducing or eliciting or helping to induce or elicit, directly or indirectly, at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity (or complement-mediated lysis), complement fixation, induction of apoptosis, induction of one or more biologically active
15 signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

 In another aspect, binding constructs of the invention are capable of induction of biologically active signals by activation or inhibition of one or more molecules selected
20 from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components. Such biologically active molecules are, for example, proteases. Other biologically active molecules are, for example, cytokines, including by way of example monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and
25 interleukins.

 In another aspect, constructs of the invention are capable of induction, or participation in the induction, of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

In another aspect, constructs of the invention are capable of induction, or participation in the induction, of one or more immune effector cells that results in antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

5 In another aspect, constructs of the invention are capable of participating in and/or initiating apoptosis within target cells, for example, by activating one or more signalling mechanisms or molecules.

In another aspect, constructs of the invention are capable of induction, or participation in the induction, of cellular activation, wherein said activation leads to changes
10 in cellular transcriptional activity. In one embodiment, cellular transcriptional activity is increased. In another embodiment, cellular transcriptional activity is decreased.

In another aspect, constructs of the invention having tail regions comprising, consisting essentially of, or consisting of, constant regions from IgA or IgE molecules, are capable of induction, or participation in the induction, of degranulation of neutrophils and/or
15 mast cells.

In another aspect, constructs of the invention are capable of promotion, or participation in the promotion, of neutralization of an infectious agent, wherein said infectious agent is, for example, a bacterium, a virus, a parasite, or a fungus.

In another aspect, constructs of the invention are capable of promoting, or
20 participating in the promotion of, neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins. Such toxins include, for example, exotoxins selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin,
25 and *Bacillus anthracis* EF toxin. Other toxins include, for example, saxitoxins, tetrodotoxin, mushroom toxins (amatoxins, gyromitrin, orellanine, *etc.*), aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.

In another aspect, constructs of the invention are capable of binding to an intracellular target to, for example, effect (or participate in effecting) a cellular function.
30 Such constructs include, for example, constructs that include a tail region comprising,

consisting essentially of, or consisting of, a native or engineered IgA CH2 domain region and a native or engineered IgA CH3 domain region, said tail region being capable of binding J chain. Such a tail region is found, for example, in the 2H7 scFv IgAH WlgACH2 WCH3 + JChain construct. Thus, the invention includes constructs having, for example, an
5 "Anti-Intracellular Target" binding domain (for example, and "Anti-Intracellular Target" scFv), a connecting region, and a native or engineered IgA constant region capable of binding J chain (for example, WlgACH2 WCH3).

In still another aspect, constructs of the invention include a molecule wherein an N-terminally immunoglobulin heavy chain constant region polypeptide comprises an IgG
10 CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

In yet another aspect, the invention includes a method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein molecule that is less than about 120kK, or less than about
15 150kD, as measured, for example, by HPLC and non-reducing gels and consists essentially of (a) a first protein or peptide molecule that is capable of binding to cells within said target cell population, and (b) a second protein or peptide molecule that is capable of (i) binding to an Fc receptor and/or (ii) inducing target cell apoptosis, and/or (iii) fixing complement, wherein said first protein or peptide molecule is directly connected to said second protein or
20 peptide molecule, or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

Various specific constructs of the invention include, by way of example only,
25 the following:

1. 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3
2. 2H7 scFv VH L11S IgE CH2 CH3 CH4
3. 2H7 scFv VH L11S mIgE CH2 CH3 CH4
4. 2H7 scFv VH L11S mIgAH WlgACH2 T4CH3
- 30 5. 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3

	6. 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3
	7. 2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3
	8. 2HU scFv VH L11S (CSS-S) H P331S CH2 WCH3
	9. 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3
5	10. 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3
	11. 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3
	12. 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3
	13. G28-1 scFv (SSS-S) H WCH2 WCH3
	14. G28-1 scFv IgAH WCH2 WCH3
10	15. G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3
	16. G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3
	17. G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3
	18. G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3
	19. CTLA4 (SSS-S) H P238SCH2 WCH32
15	20. CTLA4 (CCC-P) WH WCH2 WCH3
	21. FC2-2 scFv (SSS-S) H WCH2 WCH3
	22. FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3
	23. UCHL-1 scFv (SSS-S) H WCH2 WCH3
	24. UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3
20	25. 5B9 scFv (SSS-S) H WCH2 WCH3
	26. 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3
	27. 2H7 scFv (SSS-S) H WCH2 WCH3
	28. 2H7 scFv (SSS-S) H P238SCH2 WCH3
	29. 2H7 scFv IgAH WCH2 WCH3
25	30. 2H7 scFv IgAH WlgACH2 T4CH3
	31. 2H7 scFv IgAH WlgACH2 WCH3 + JChain
	32. 2H7 scFv (CCC-P) WH WCH2 WCH3
	33. 2H7 scFv (SSS-S) H WCH2 F405YCH3
	34. 2H7 scFv (SSS-S) H WCH2 F405ACH3
30	35. 2H7 scFv (SSS-S) H WCH2 Y407ACH3

	36. 2H4 scFv (SSS-S) HWCH2 F405A, Y407ACH3
	37. 2H7 _{scFv} (CSS-S) H WCH2 WCH3
	38. 2H7 scFv (SCS-S) H WCH2 WCH3
	39. 2H7 scFv (SSC-P) H WCH2 WCH3
5	40. 2H7 scFv (CSC-S) H WCH2 WCH3
	41. 2H7 scFv (CCS-P) H WCH2 WCH3
	42. 2H7 scFv (SCC-P) H WCH2 WCH3
	43. 2H7 scFv VH L11S (SSS-S) H WCH2 WCH3
	44. 2H7 scFv VH L11S (CSS-S) H WCH2 WCH3
10	45. G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3
	46. G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3
	47. G28-1 scFv VH L11S (SCC-P) H WCH2 WCH3
	48. G28-1 scFv VH L11S mIgE CH2 CH3 CH4
	49. G28-1 scFv VH L11S mIgAH WlgACH2 T4CH3
15	50. G28-1 scFv VH L11S hIgE CH2 CH3 CH4
	51. G28-1 scFv VH L11S hIgAH WlgACH2 T4CH3
	52. HD37 scFv IgAH WCH2 WCH3
	53. HD37 scFv (SSS-S) H WCH2 WCH3
	54. HD37 scFv VH L11S (SSS-S) H WCH2 WCH3
20	55. L6 scFv IgAH WCH2 WCH3
	56. L6 scFv VHL11S (SSS-S) H WCH2 WCH3
	57. 2H7 scFv-llama IgG1
	58. 2H7 scFv-llama IgG2
	59. 2H7 scFv-llama IgG3
25	60. CD16-6 low (ED)(SSS-S) H P238SCH2 WCH3
	61. CD16-9 high (ED)(SSS-S) H P238SCH2 WCH3
	62. 2e12 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
	63. 10A8 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
	64. 40.2.36 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
30	65. 2H7 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT

	66. G19-4 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
	67. 2e12 scFv (SSS-s)H WCH2 WCH3—hCD80TM/CT
	68. 2e12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT
	69. 2e12 scFv IgE CH2CH3CH4—hCD80TM/CT
5	70. 2e12 scFv (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT
	71. 2e12 scFv (SSS-s)H WCH2 WCH3—mFADD-TM/CT
	72. 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp3-TM/CT
	73. 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT
	74. 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp8--TM/CT
10	75. 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT
	76. 2e12 scFv (SSS-s)H WCH2 WCH3—hcasp3--TM/CT
	77. 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp3-TM/CT
	78. 2e12 scFv (SSS-s)H WCH2 WCH3—hcasp8--TM/CT
	79. 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp8-TM/CT
15	80. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
	81. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hCD80TM/CT
	82. 1D8 scFv—mIgAT4—hCD80TM/CT
	83. 1D8 scFv--hIgE—hCD80TM/CT
	84. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT
20	85. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mFADD-TM/CT
	86. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasp3-TM/CT
	87. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT
	88. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasp8--TM/CT
	89. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT
25	90. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcasp3--TM/CT
	91. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hcasp3-TM/CT
	92. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcasp8--TM/CT
	93. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hcasp8-TM/CT L6 scFv (SSS-S) H WCH2 WCH3
30	94. 2H7 scFv CD154 (L2)

95. 2H7 scFv CD154 (S4)
96. CTLA4 IgAH IGACH2CH3
97. CTLA4 IgAH IgACH2 T4CH3
98. 2H7 scFv IgAH IgACH2CH3
- 5 99. 2H7 scFv IgAH IgAHCH2 T18CH3
100. 2H&-40.2.220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-cd20-
anti-cd40)
101. 2H7 scFv IgAH IgACH2 T4CH3-hCD89 TM/CT
102. G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT
- 10 103. 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

These and other aspects of the present invention will become further apparent upon reference to the following detailed description and attached drawings. As noted herein, all referenced patents, articles, documents, and other materials disclosed or identified herein are hereby incorporated by reference in their entireties as if each was incorporated
15 individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows DNA and deduced amino acid sequences [SEQ ID NOS:___] of 2H7scFv-Ig, a binding domain-immunoglobulin fusion protein capable of specifically
20 binding CD20.

Figure 2 shows production levels of 2H7 scFv-Ig by transfected, stable CHO lines and generation of a standard curve by binding of purified 2H7 scFv-Ig to CHO cells expressing CD20.

Figure 3 shows SDS-PAGE analysis of multiple preparations of isolated
25 2H7scFv-Ig protein.

Figure 4 shows complement fixation (Fig. 4A) and mediation of antibody-dependent cellular cytotoxicity (Fig. 4B) by 2H7scFv-Ig.

Figure 5 shows the effect of simultaneous ligation of CD20 and CD40 on growth of normal B cells.

Figure 6 shows the effect of simultaneous ligation of CD20 and CD40 on CD95 expression and induction of apoptosis in a B lymphoblastoid cell line.

Figure 7 shows DNA and deduced amino acid sequences [SEQ ID NOS: __] of 2H7scFv-CD154 L2 (Fig. 7A, SEQ ID NOS: __) and 2H7scFv-CD154 S4 (Fig. 7B, SEQ ID NOS: __) binding domain-immunoglobulin fusion proteins capable of specifically binding CD20 and CD40.

Figure 8 shows binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion proteins to CD20+ CHO cells by flow immunocytometry.

Figure 9 shows binding of Annexin V to B cell lines Ramos, BJAB, and T51 after binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion protein to cells.

Figure 10 shows effects on proliferation of B cell line T51 following binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion protein.

Figure 11 depicts schematic representations of the structures of 2H7ScFv-Ig fusion proteins [SEQ ID NOS: __] referred to as CytoxB or CytoxB derivatives: CytoxB-MHWTG1C (2H7 ScFv, mutant hinge, wild-type human IgG1 Fc domain), CytoxB-MHMG1C (2H7 ScFv, mutant hinge, mutated human IgG1 Fc domain) and CytoxB-IgAHWTHG1C (2H7 ScFv, human IgA-derived hinge [SEQ ID NO: __], wild-type human IgG1 Fc domain). Arrows indicate position numbers of amino acid residues believed to contribute to FcR binding and ADCC activity (heavy arrows), and to complement fixation (light arrows). Note absence of interchain disulfide bonds.

Figure 12 shows SDS-PAGE analysis of isolated CytoxB and 2H7scFv-CD154 binding domain-immunoglobulin fusion proteins.

Figure 13 shows antibody dependent cell-mediated cytotoxicity activity of CytoxB derivatives.

Figure 14 shows complement dependent cytotoxicity of CytoxB derivatives.

Figure 15 shows serum half-life determinations of CytoxB-MHWTG1C in macaque blood samples.

Figure 16 shows effects of CytoxB-MHWTG1C on levels of circulating CD40+ B cells in macaque blood samples.

Figure 17 shows production levels of HD37 (CD19-specific) ScFv-Ig by transfected mammalian cell lines and generation of a standard curve by binding of purified HD37 ScFv-Ig to cells expressing CD19.

Figure 18 shows production levels of L6 (carcinoma antigen) ScFv-Ig by transfected, stable CHO lines and generation of a standard curve by binding of purified L6 ScFv-Ig to cells expressing L6 antigen.

Figure 19 shows antibody dependent cell-mediated cytotoxicity activity of binding domain-immunoglobulin fusion proteins 2H7 ScFv-Ig, HD37 ScFv-Ig and G28-1 (CD37-specific) ScFv-Ig.

Figure 20 shows antibody dependent cell-mediated cytotoxicity activity of L6 ScFv-Ig fusion proteins.

Figure 21 shows SDS-PAGE analysis of L6 ScFv-Ig and 2H7 ScFv-Ig fusion proteins.

Figure 22 shows SDS-PAGE analysis of G28-1 ScFv-Ig and HD37 ScFv-Ig fusion proteins.

Figure 23 presents a sequence alignment of immunoglobulin hinge and CH2 domains of human IgG1 (SEQ ID NO:__) with the hinge and CH2 domains of llama IgG1 (SEQ ID NO:__), IgG2 (SEQ ID NO:__), and IgG3 (SEQ ID NO:__).

Figure 24 illustrates migration of purified 2H7 scFv llama IgG fusion proteins in a 10% SDS polyacrylamide gel. Purified fusion proteins (5 μ g per sample) were prepared in non-reducing sample buffer (lanes 2-5) and in reducing sample buffer (lanes 6-9). Lane 1: molecular weight markers (non-reduced); lanes 2 and 6: 2H7 scFv-llama IgG1 (SEQ ID NO:__); Lanes 3 and 7: 2H7 scFv-llama IgG2 (SEQ ID NO:__); lanes 4 and 8: 2H7 scFv-llama IgG3 (SEQ ID NO:__); and Lanes 5 and 9: Rituximab (chimeric anti-CD20 antibody (human IgG1 constant region)).

Figure 25 shows binding of 2H7 scFv-llama IgG1 (SEQ ID NO:__), 2H7 scFv-llama IgG2 (SEQ ID NO:__), and 2H7 scFv-llama IgG3 (SEQ ID NO:__) to CD20+ CHO cells detected by flow immunocytometry.

Figure 26 depicts CDC activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv-llama IgG1 (SEQ ID NO:__), 2H7 scFv-llama IgG2 (SEQ ID NO:__), and 2H7 scFv-

llama IgG3 (SEQ ID NO:__), and 2H7 scFv human IgG1 (2H7 scFv IgG WTH WTCH2CH3) (SEQ ID NO:__) against BJAB cells in the presence of rabbit complement. Rituximab was included as a control.

Figure 27 shows antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv-llama IgG1 (SEQ ID NO:__), 2H7 scFv-llama IgG2 (SEQ ID NO:__), and 2H7 scFv-llama IgG3 (SEQ ID NO:__). Effector cells (human PBMC) were combined with target cells (BJAB cells) at three different ratios, 1:25, 1:50, and 1:100. Rituximab was included as a control. Each data point represents three separate measurements.

Figure 28 shows antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv-llama IgG1 (SEQ ID NO:__), 2H7 scFv-llama IgG2 (SEQ ID NO:__), and 2H7 scFv-llama IgG3 (SEQ ID NO:__). Effector cells (llama PBMC) were combined with target cells (BJAB cells) at three different ratios, 1:25, 1:50, and 1:100. Rituximab was included as a control. Each data point represents three separate measurements.

Figure 29 depicts complement dependent cytotoxicity activity of Reh cells (acute lymphocytic leukemia) expressing scFv-Ig fusion proteins on the cell surface. Reh cells were transfected with constructs encoding scFv antibodies specific for human costimulatory molecules, CD152, CD28, CD40, and CD20, fused to human IgG1 wild-type hinge-CH2-CH3, which was fused to human CD80 transmembrane and cytoplasmic tail domains. Complement dependent cytotoxicity activity was measured in the presence and absence of rabbit complement (plus C' and no C', respectively). The data represent the average of duplicate samples. Reh anti-hCD152 scFvIg: Reh cells transfected with polynucleotide 10A8 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:__); Reh anti-hCD28scFvIg: 2E12 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:__); Reh anti-hCD40scFvIg: 4.2.220 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:__); and Reh anti-hCD20scFvIg: 2H7 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:__).

Figure 30 presents antibody dependent cell-mediated cytotoxicity activity of Reh cells that were transfected with constructs encoding scFv antibodies specific for human costimulatory molecules, CD152, CD28, CD40, and CD20, as described for Figure 29, and

for murine CD3, fused to human mutant IgG1 hinge and mutant CH2 and wild type CH3 (Reh anti-mCD3scFv designating Reh cells transfected with polynucleotide 500A2 scFv IgG MTH (SSS) MTCH2WTCH3 SEQ ID NO:__), which was fused to human CD80 transmembrane and cytoplasmic tail domains. The data represent the average of quadruplicate samples.

Figure 31 lists immunoglobulin constant region constructs that were used in experiments illustrated in subsequent figures.

Figure 32 depicts complement dependent cytotoxicity activity of CTLA-4 Ig fusion proteins, CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:__) (2 μ g/ml) and CTLA-4 IgG MTH MTCH2WTCH3 (SEQ ID NO:__) (2 μ g/ml), in the presence and absence of rabbit complement (plus C' and no C', respectively). The target cells were Reh cells and Reh cells transfected with CD80 (Reh CD80.10).

Figure 33 shows antibody dependent cell-mediated cytotoxicity activity of CTLA-4 Ig fusion proteins, CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:__) (2 μ g/ml) and CTLA-4 IgG MTH MTCH2WTCH3 (SEQ ID NO:__) (2 μ g/ml). Effector cells, human PBMC, were added to target cells, Reh or Reh CD80.1, at the ratios indicated. Figure 33A presents the level of natural killing in Reh CD80.1 cells in the absence of any Ig fusion protein. Figure 33B presents antibody dependent cell-mediated cytotoxicity mediated by CTLA-4 IgG MTH MTCH2WTCH3, and Figure 33C presents antibody dependent cell-mediated cytotoxicity mediated by CTLA-4 IgG WTH (CCC) WTCH2CH3. Each data point represents the average percent specific killing measured in four sample wells.

Figure 34 illustrates binding of 2H7 (anti-CD20) scFv Ig fusion proteins to (CD20+) CHO cells by flow immunocytofluorimetry.

Figure 35 presents an immunoblot of 2H7 scFv IgG and IgA fusion proteins. COS cells were transiently transfected with various 2H7 scFv Ig fusion protein constructs. The expressed polypeptides were immune precipitated with protein A, separated in a non-reducing SDS polyacrylamide gel, and then transferred to a polyvinyl fluoride membrane. Proteins were detected using an anti-human IgG (Fc specific) horseradish peroxidase conjugate. Lane 1: vector only; lane 2: 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:__); lane 3: 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:__); lane 4: 2H7

scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:__); lane 5: 2H7 scFv IgAH IgG WTCH2CH3 (SEQ ID NO:__); and lane 6: 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:__).

Figure 36 illustrates binding of 2H7 scFv IgAH IgACH2CH3 polypeptide (SEQ ID NO:__) and 2H7 scFv IgAH IgAT4 (SEQ ID NO:__) to (CD20+) CHO cells by flow immunocytofluorimetry. The source of the polypeptides was culture supernatants from transiently transfected COS cells. COS cells transfected with a plasmid comprising a sequence encoding 2H7 scFv IgAH IgACH2CH3 were co-transfected with a plasmid containing nucleotide sequence encoding human J chain.

Figure 37 illustrates antibody dependent cell-mediated cytotoxicity activity of anti-CD20 (2H7) scFv Ig fusion proteins against BJAB target cells using whole blood as the source of effector cells. Purified 2H7 scFv Ig fusion proteins were titrated and combined with ⁵¹Cr-labeled BJAB cells (5×10^4) and whole blood (1:4 final dilution). Each data point represents the average percent specific killing measured in four sample wells.

Figure 38 demonstrates antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv Ig fusion proteins (5 μ g/ml) against ⁵¹Cr-labeled BJAB cells at 0.25, 0.125, and 0.625 dilutions of whole blood. Each data point represents the average percent specific killing measured in four sample wells.

Figure 39 shows a comparison of antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv IgG MTH (SSS) WTCH2CH3 (5 μ g/ml) and 2H7 scFv IgAH IgACH2CH3 (5 μ g/ml) when human PBMC are the source of effector cells (Figure 39A) and when human whole blood is the source of effector cells (Figure 39B).

Figure 40 presents an immunoblot of 2H7 scFv IgG fusion proteins. COS cells were transiently transfected with various 2H7 scFv Ig fusion protein constructs. Culture supernatants containing the expressed polypeptides were separated in a non-reducing SDS polyacrylamide gel, and then were transferred to a polyvinyl fluoride membrane. Proteins were detected using an anti-human IgG (Fc specific) horseradish peroxidase conjugate. Lanes 1-5: purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 at 40 ng, 20 ng, 10 ng/ 5 ng, and 2.5 ng per lane, respectively. Culture supernatants were separated in lanes 6-9. Lane 6: 2H7 scFv IgG WTH (CCC) WTCH2CH3; lane 7: 2H7 scFv IgG MTH

(CSS) WTCH2CH3; lane 8: 2H7 scFv IgG MTH (SCS) WTCH2CH3; and lane 9: 2H7 scFv VHSE11 IgG MTH (SSS) WTCH2CH3. The molecular weight (kDa) of marker proteins is indicated on the left side of the immunoblot.

Figure 41 illustrates cell surface expression of 1D8 (anti-murine 4-1BB) scFv IgG WTH WTCH2CH3-CD80 fusion protein on K1735 melanoma cells by flow immunofluorimetry (Fig. 41A). The scFv fusion protein was detected with phycoerythrin-conjugated F(ab')₂ goat anti-human IgG. Fig. 41B depicts growth of tumors in naïve C3H mice transplanted by subcutaneous injection with wild type K1735 melanoma cells (K1735-WT) or with K1735 cells transfected with 1D8 scFv IgG WTH WTCH2CH3-CD80 (K1735-1D8). Tumor growth was monitored by measuring the size of the tumor. Fig. 41C demonstrates the kinetics of tumor growth in naïve C3H mice injected intraperitoneally with monoclonal antibodies to remove CD8⁺, CD4⁺, or both CD4⁺ and CD8⁺ T cells prior to transplantation of the animals with K1735-1D8 cells.

Figure 42 demonstrates therapy of established K1735-WT tumors using K1735-1D8 as an immunogen. Six days after mice were transplanted with K1735-WT tumors, one group (five animals) was injected subcutaneously with K1735-1D8 cells (open circles) or irradiated K1735-WT cells (solid squares) on the contralateral side. A control group of mice received PBS (open squares). Treatments were repeated on the days indicated by the arrows.

Figure 43 shows the growth of tumors in animals that were injected subcutaneously with 2×10^6 K1735-WT cells (solid squares) and the growth of tumors in animals that were injected subcutaneously with 2×10^6 K1735-WT cells plus 2×10^5 K1735-1D8 cells (open triangles).

Figure 44 presents a flow cytometry analysis of antigen104 murine sarcoma tumor cells transfected with 1D8 scFv IgG WTH WTCH2CH3-CD80 isolated after repeated rounds of panning against anti-human IgG. Transfected cells expressing 1D8 scFv IgG WTH WTCH2CH3-CD80 were detected with fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (depicted in black). Untransfected cells are shown in gray.

Figure 45 illustrates migration of various 2H7 scFv Ig fusion proteins in a 10% SDS-PAGE gel. 2H7 was the anti-CD20 scFv and 40.2.220 was the anti-CD40 scFv.

Lane 1: Bio-Rad prestained molecular weight standards; lane 2: anti-CD20 scFv IgG MTH (SSS) MTCH2WTCH3; lane 3: anti-CD20 scFv IgG MTH (SSS) WTCH2CH3; lane 4: 2H7 scFv IgAH IgG WTCH2CH3; lane 5: anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3; lane 6: Rituximab; lane 7: Novex Multimark® molecular weight standards.

Figure 46 illustrates effector function as measured in an antibody dependent cell-mediated cytotoxicity assay of 2H7 Ig fusion proteins that contain a mutant CH2 domain or wild type CH2 domain. The percent specific killing of BJAB target cells in the presence of human PBMC effector cells by 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (diamonds) was compared to 2H7 scFv IgG MTH (SSS) WTCH2CH3 (squares) and 2H7 scFv IgAH IgG WTCH2CH3 (triangles) and Rituximab (circles).

Figure 47 shows cell surface expression of an anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 (SEQ ID NO:__) fusion protein on Reh cells (Fig. 47A) and T51 lymphoblastoid cells (Fig. 47B) by measuring the linear fluorescent equivalent (LFE) using flow immunocytofluorimetry.

Figure 48 presents the percent specific killing of untransfected Reh and T51 cells and the percent specific killing of Reh cells (Reh anti-hCD3) (Fig. 48A) and T51 cells (T51 anti-hCD3) (Fig. 48B) that were transfected with a construct encoding scFv antibodies specific for human CD3, fused to human IgG1 wild-type hinge-CH2-CH3, which was fused to human CD80 transmembrane and cytoplasmic tail domains (anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 (SEQ ID NO:___). Human PBMC (effector cells) were combined with BJAB target cells at the ratios indicated.

Figure 49 illustrates binding of 5B9, an anti-murine CD137 (4-1BB) monoclonal antibody, and a 5B9 scFv IgG fusion protein (5B9 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:___) to stimulated human PBMC. Binding of the 5B9 scFv IgG fusion protein was detected by flow immunocytofluorimetry using FITC conjugated goat anti-human IgG. Binding of the 5B9 monoclonal antibody was detected with FITC conjugated goat anti-mouse IgG.

Figure 50 illustrates the effect of the LV_H11S mutation on the expression of 2H7 LV_H11S scFv WCH2 WCH3 ("CytosB scFv Ig"; SEQ ID NO:___) in CHO cell lines.

Figure 51 shows a semi-quantitative SDS-PAGE analysis examining the expression of 2H7 LV_{H11S} scFv WCH2 WCH3 (SEQ ID NO:__) when transiently transfected in CHO cells. Lanes 2-5 are various amounts of 2H7 LV_{H11S} scFv WCH2 WCH3. Lanes 6-10 are 10µl samples from five different clones expressing 2H7 LV_{H11S} scFv WCH2 WCH3.

Figure 52 shows differences in binding capacity between a G28-1 LV_{H11S} scFv Ig construct (SEQ ID NO: __) and a G28-1 wild type scFv Ig binding domain fusion protein construct (SEQ ID NO:__), both obtained from transiently transfected COS cells. Binding to Ramos cells was determined using flow cytometry. The data illustrates a significant increase in binding of the V_{H11S} protein to CD37+Ramos cells.

Figure 53 illustrates increased levels of expression of a G28-1 LV_{H11S} scFv Ig construct (SEQ ID NO: __) compared to a G28-1 wild type scFv Ig construct in COS. Protein levels were compared using immunoblot analysis. Both immunoblot gels have quantitated amounts of purified a G28-1 scFv Ig (SSS-S) H WCH2 WCH3 construct of the invention in lanes 1-4. Lanes 5-9 of the first immunoblot represent five different clones each transfected with G28-1 scFv (SSS-S) H WCH2 WCH3, while lanes 5-9 of the second immunoblot represent five different clones transfected with G28-1 LV_{H11S} scFv (SSS-S) H WCH2 WCH3. The immunoblots illustrate that the LV_{H11S} form causes the G28-1 scFv Ig construct to express at very high levels.

Figure 54 illustrates the binding of 2H7 scFv Ig derivatives with altered hinges (SEQ ID NOs: __, __, __, __, __, __, __) to CHO cells expressing CD20 (CD20+ CHO) by flow cytometry, and indicates that these altered connecting region hinge constructs (including (SSS-S), (CSS-S), (SCS-S) and (CSC-S) hinge regions) retain binding function to CD20.

Figure 55 shows the ability to mediate antibody dependent cell-mediated cytotoxicity of various constructs against Bjab targets: (A) 2H7 scFv Ig constructs of the invention that contain connecting regions comprising (CSS-S), (SCS-S), (CSC-S), and (SSS-S) hinges (SEQ ID NOs: __, __, __, __, __, __, __) and (B) 2H7 scFv constructs of the invention with various connecting regions and tail regions (SEQ ID NOs: __, __, __, __, __, __).

Percent specific killing is compared to total killing induced by a detergent. The controls are

natural killing in target cells with effectors added and a 2H7 construct with an IgA hinge connecting region and IgA-derived tail region that does not bind PBMC effectors.

Figure 56 illustrates the ability of various 2H7 scFv Ig constructs of the invention (SEQ ID NO: __, __, __, __, __) that include connecting regions having various hinge regions (*e.g.*, (CSC-S), (SSS-S), (SCS-S), and (CSS-S)) to mediate complement activity in Ramos cells. Percent specific killing is measured against the control of complement only, and 100% killing was determined by exposure of cells to detergent.

Figure 57 illustrates the shows the binding of 2H7 scFv Ig constructs of the invention containing different tail regions (SEQ ID NO: __, __, __) to CD20+ CHO using immunocytofluorimetry. The different proteins were detected using FITC conjugated to anti-IgG, anti-IgA, and anti-IgE.

Figure 58A shows the binding of 2H7 V_H L11S scFv IgE_H2CH3CH4, purified using Hydrophobic charge induction chromatography (HCIC) and eluted at different pHs 4.0 and 3.5, (SEQ ID NO: __) in CD20+ CHO cells by flow cytometry, indicating that the proteins bound CD20 whether eluted at pH 4.0 or 3.5. **Figure 58B** is a data graph indicating the ability of these 2H7 V_H L11S scFv IgE constructs of the invention to mediate, for example, ADCC in Bjab target cells.

Figure 59 shows the binding capacity of G28-1 V_H L11S mIgE_H2CH3CH4 (SEQ ID NO: __) (A) to Bjab and Ramos target cells and (B) to CD20+ CHO cells by flow cytometry.

Figure 60 shows the High Performance Liquid Chromatography (HPLC) profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H (P238S)CH2 WCH3 (SEQ ID NO: __) (B) 2H7 scFv (CSS-S) H WCH2 WCH3, (SEQ ID NO: __) (C) 2H7 scFv (SCS-S) H WCH2 WCH3, (SEQ ID NO: __) and (D) 2H7 scFv (SSS-S) H WCH2 (Y407A)CH3 (SEQ ID NO: __), indicating that construct A has apparent molecular weight forms of 100kD and 75kD and that, by introducing certain changes a predominant 75kD molecular weight form is obtained, as seen in constructs B, C, and D. See Example 40.

Figure 61 shows the HPLC profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (SEQ ID NO: __) (B) 2H7 scFv (CSC-S) H WCH2 WCH3, (SEQ ID NO: __) (C) 2H7 scFv (CCC-P) H WCH2 WCH3, (SEQ ID

NO: __) and (D) 2H7 scFv IgAH WCH2 WCH3 (SEQ ID NO: __), indicating that construct A has apparent molecular weight forms of 100kD and 75kD and that, by introducing certain changes a predominant 75kD molecular weight form is obtained, as seen in constructs B and C, while construct D (which has an IgA tail region) has an apparent molecular weight of 150kD. See Example 40.

Figure 62 shows the HPLC profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (SEQ ID NO: __) (B) 2H7 scFv (SCS-S) H WCH2 WCH3, (SEQ ID NO: __) (C) 2H7 scFv IgA 3TCH2 WCH3, (SEQ ID NO: __) and (D) 2H7 scFv (SSS-S) H WCH2 (F405A Y407A)CH3 (SEQ ID NO: __), indicating that construct A has two forms with apparent molecular weights at 100kD and 75kD, construct B has a predominant form with an apparent molecular weight of 75kD, while construct C with a T4 mutation leads to three forms with apparent molecular weights near 600kD and construct D with a double point mutation in the CH3 region leads to a predominant form having an apparent molecular weight less than 44kD. A T4 mutation here refers to a truncation of four amino acids from a CH3 region. See Example 40.

Figure 63 compares the effect on binding CD20+CHO cells by 2H7 V_H L11S scFv Ig constructs (SEQ ID NOs: __,__), with and without F405A and Y407A alterations in the CH3 region, by flow cytometry, indicating a loss of binding capability with this double amino acid change. See Example 41.

Figure 64 shows the binding capacity of FITC conjugated 2H7 V_H L11S scFv Ig derivatives (SEQ ID NOs: __,__,__,__) to CH20+ CHO cells by flow cytometry, indicating that these constructs do not lose binding capacity when conjugated to a fluorescent marker. See Example 41.

Figure 65 shows a nonreducing SDS-PAGE analysis examining 10 µg (per lane) of various purified 2H7 V_H L11S scFv Ig constructs of the invention (SEQ ID NOs: __,__,__,__,__,__), indicating an apparent molecular weight for each construct in reference to a standard molecular weight marker in lane 1. See Example 41.

Figure 66 compares the CH2 domain sequences of four different human IgG regions, hIgG1, hIgG2, hIgG3, hIgG4, and one rat region, rIgG2b. Point mutations affecting ADCC and CDC are labeled with arrows. See Example 52.

Figure 67 demonstrates the ability of various 2H7 V_H L11S scFv Ig constructs (SEQ ID NOs: __,__) to mediate ADCC in CHO and Lec13 CHO transiently transfected cells, indicating that constructs expressed in Lec 13 CHO cells had a 20% increase in specific killing over the same construct expressed in regular CHO cells. See Example 42.

Figure 68 shows SDS-PAGE analysis, both reduced and nonreduced, of high and low affinity alleles of soluble CD 16(ED) (SSS-S) H P283S CH2 WCH3 (SEQ ID NOs: __,__). See Example 43.

Figure 69 demonstrates the different binding capabilities of the high and low affinity CD16 fusion proteins (SEQ ID NOs: __,__) to 2H7 V_H L11S scFv (CSC-S) WCH2 WCH3 (SEQ ID NO: __) or 2H7 V_H L11S scFv (SSS-S) WCH2 WCH3 (SEQ ID NO: __), and indicates a loss of high and low affinity allele binding using P238S CH2 constructs. See Example 43.

Figure 70 shows a diagram of (A) an assay used to detect changes in Fc receptor binding using FITC conjugated CD16 extracellular domain Ig fusion protein with a mutated tail, which eliminates self-association and B) a mammalian display system using cell surface expression of SMIPs. See Example 44.

Figure 71 shows the induction of apoptosis in Bjab and Ramos cells by various mAbs (SEQ ID NOs: __,__,__,__,__,__,__) and scFvIg constructs of the invention (SEQ ID NOs: __,__,__,__,__,__,__). See Example 45.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel molecules useful, for example, as therapeutics, as well as for other purposes including diagnostic and research purposes. Such molecules have, for example, antigen-binding or other binding function(s) and, for example, one or more effector functions. The invention includes molecular constructs, including binding domain-immunoglobulin fusion proteins, and related compositions and methods, which will be useful in immunotherapeutic and immunodiagnostic applications, and in research methods, and which offer certain advantages over antigen-specific compounds and polypeptides of the prior art. The constructs, including fusion proteins, of the present

invention are preferably single polypeptide chains that comprise, in pertinent part, the following fused or otherwise connected domains or regions: a binding region construct, such as a binding domain or polypeptide, a connecting region construct including, for example, a native or engineered immunoglobulin hinge region polypeptide, and a tail region construct, including, for example, a construct that may comprise, consist essentially of, or consist of, a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide. According to certain embodiments that are particularly useful for gene therapy, the constructs, including fusion proteins, of the present invention may further comprise a native or engineered plasma membrane anchor domain. According to certain other preferred embodiments the constructs, including fusion proteins, of the present invention may further include a tail region having a native or engineered immunoglobulin heavy chain CH4 constant region polypeptide. In particularly preferred embodiments, the binding regions, such as polypeptide domains, of which the constructs, including binding domain-immunoglobulin fusion proteins, are comprised are, or are derived from, polypeptides that are the products of human gene sequences, but the invention need not be so limited and may in fact relate to constructs, including binding domain-immunoglobulin fusion proteins, as provided herein that are derived from any natural or artificial source, including genetically engineered and/or mutated polypeptides.

The present invention relates in part to the surprising observation that the novel constructs, including binding domain-immunoglobulin fusion proteins, described herein are capable of immunological activity. More specifically, these proteins retain the ability to participate in well known immunological effector activities including, for example, antibody dependent cell mediated cytotoxicity (*e.g.*, subsequent to antigen binding on a cell surface, engagement and induction of cytotoxic effector cells bearing appropriate Fc receptors, such as Natural Killer cells bearing FcR γ III, under appropriate conditions) and/or complement fixation in complement dependent cytotoxicity (*e.g.*, subsequent to antigen binding on a cell surface, recruitment and activation of cytolytic proteins that are components of the blood complement cascade) despite having structures not be expected to be capable of promoting such effector activities or to promotion of such activities as

described herein. For reviews of ADCC and CDC see, *e.g.*, Carter, 2001 *Nat. Rev. Canc.* 1:118; Sulica *et al.*, 2001 *Int. Rev. Immunol.* 20:371; Maloney *et al.*, 2002 *Semin. Oncol.* 29:2; Sondel *et al.*, 2001 *Hematol Oncol Clin North Am* 15(4):703-21; Maloney 2001 *Anticanc. Drugs* 12 Suppl.2:1-4. IgA activation of complement by the alternative pathway is described, for example, in Schneiderman *et al.*, 1990 *J. Immunol.* 145:233.. As described in greater detail below, ADCC, complement fixation, and CDC are unexpected functions for constructs, including fusion proteins, comprising for example immunoglobulin heavy chain regions and having the structures described herein, and in particular for immunoglobulin fusion proteins comprising, for example, immunoglobulin hinge region polypeptides that are compromised in their ability to form interchain, homodimeric disulfide bonds.

Another advantage afforded by the present invention is constructs, including binding domain-immunoglobulin fusion polypeptides, of the invention that can be produced in substantial quantities that are typically greater than those routinely attained with single-chain antibody constructs of the prior art, for example. In preferred embodiments, constructs, including the binding domain-immunoglobulin fusion polypeptides, of the present invention are recombinantly expressed in mammalian or other desired and useful expression systems, which offer the advantage of providing polypeptides that are stable *in vivo* (*e.g.*, under physiological conditions). According to non-limiting theory, such stability may derive in part from posttranslational modifications, and specifically glycosylation. Production of the constructs, including binding domain-immunoglobulin fusion protein constructs, of the invention via recombinant mammalian expression has been attained in static cell cultures at a level of greater than 50 mg protein per liter culture supernatant and has been routinely observed in such cultures at 10-50 mg/liter, such that preferably at least 10-50 mg/liter may be produced under static culture conditions; also contemplated are enhanced production, in whole or in part, of the protein constructs of the invention using art-accepted scale-up methodologies such as "fed batch" (*i.e.*, non-static) production, where yields of at least 5-500 mg/l, and in some instances at least 0.5-1 gm/l, depending on the particular protein product, are obtained.

A construct, including a binding domain polypeptide, according to the present invention may be, for example, any polypeptide that possesses the ability to

specifically recognize and bind to a cognate biological molecule or complex of more than one molecule or assembly or aggregate, whether stable or transient, of such a molecule. Such molecules include proteins, polypeptides, peptides, amino acids, or derivatives thereof; lipids, fatty acids or the like, or derivatives thereof; carbohydrates, saccharides or the like or derivatives thereof; nucleic acids, nucleotides, nucleosides, purines, pyrimidines or related molecules, or derivatives thereof, or the like; or any combination thereof such as, for example, glycoproteins, glycopeptides, glycolipids, lipoproteins, proteolipids; or any other biological molecule that may be present in a biological sample. Biological samples may be provided, for example, by obtaining a blood sample, biopsy specimen, tissue explant, organ culture, biological fluid or any other tissue or cell or other preparation from a subject or a biological source. The subject or biological source may, for example, be a human or non-human animal, a primary cell culture or culture adapted cell line including but not limited to genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiable cell lines, transformed cell lines and the like, *etc.* In certain preferred embodiments of the invention, the subject or biological source may be suspected of having or being at risk for having a disease, disorder or condition, including a malignant disease, disorder or condition or a B cell disorder, which in certain further embodiments may be an autoimmune disease, and in certain other embodiments of the invention the subject or biological source may be known to be free of a risk or presence of such disease, disorder or condition.

A binding region, including a binding domain polypeptide, for example, may be any naturally occurring, synthetic, semi-synthetic, and/or recombinantly produced binding partner for a biological or other molecule that is a target structure of interest, herein sometimes referred to as an “antigen” but intended according to the present disclosure to encompass any target biological or other molecule to which it is desirable to have the subject invention, for example, a fusion protein, bind or specifically bind. Constructs of the invention, including binding domain-immunoglobulin fusion proteins, are defined to be “immunospecific” or capable of binding to a desired degree, including specifically binding, if they bind a desired target molecule such as an antigen as provided herein, at a desired level, for example, with a K_a of greater than or equal to about 10^4 M^{-1} , preferably of greater than or equal to about 10^5 M^{-1} , more preferably of greater than or equal to about 10^6 M^{-1} and still more preferably of greater than or equal to about 10^7 M^{-1} . Affinities of even greater than about 10^7 M^{-1} are still more preferred, such as affinities equal to or greater than

about about 10^7 M⁻¹, about 10^8 M⁻¹, and about 10^9 M⁻¹, and about 10^{10} M⁻¹. Affinities of binding domain-immunoglobulin fusion proteins according to the present invention can be readily determined using conventional techniques, for example those described by Scatchard *et al.*, 1949 *Ann. N.Y. Acad. Sci.* **51**:660. Such determination of fusion protein
5 binding to target antigens of interest can also be performed using any of a number of known methods for identifying and obtaining proteins that specifically interact with other proteins or polypeptides, for example, a yeast two-hybrid screening system such as that described in U.S. Patent No. 5,283,173 and U.S. Patent No. 5,468,614, or the equivalent.

Preferred embodiments of the subject invention constructs, for example,
10 binding domain-immunoglobulin fusion proteins, comprise binding regions or binding domains that may include, for example, at least one native or engineered immunoglobulin variable region polypeptide, such as all or a portion or fragment of a native or engineered heavy chain and/or a native or engineered light chain V-region, provided it is capable of binding or specifically binding an antigen or other desired target structure of interest at a
15 desired level of binding and selectivity. In other preferred embodiments the binding region or binding domain comprises, consists essentially of, or consists of, a single chain immunoglobulin-derived Fv product, for example, and scFv, which may include all or a portion of at least one native or engineered immunoglobulin light chain V-region and all or a portion of at least one native or engineered immunoglobulin heavy chain V-region, and a
20 linker fused or otherwise connected to the V-regions; preparation and testing such constructs are described in greater detail herein. Other preparation and testing methods are well known in the art.

As described herein and known in the art, immunoglobulins comprise products of a gene family the members of which exhibit a high degree of sequence
25 conservation. Amino acid sequences of two or more immunoglobulins or immunoglobulin domains or regions or portions thereof (*e.g.*, V_H domains, V_L domains, hinge regions, CH2 constant regions, CH3 constant regions) may be aligned and analyzed. Portions of sequences that correspond to one another may be identified, for instance, by sequence homology. Determination of sequence homology may be determined with any of a number
30 of sequence alignment and analysis tools, including computer algorithms well known to those of ordinary skill in the art, such as Align or the BLAST algorithm (Altschul, 1991 *J. Mol. Biol.* **219**:555-565; Henikoff and Henikoff, 1992 *Proc. Natl. Acad. Sci. USA* **89**:10915-

10919), which is available at the NCBI website (<http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST>). Default parameters may be used.

Portions, for example, of a particular immunoglobulin reference sequence and of any one or more additional immunoglobulin sequences of interest that may be compared to a reference sequence. “Corresponding” sequences, regions, fragments or the like, may be identified based on the convention for numbering immunoglobulin amino acid positions according to Kabat, *Sequences of Proteins of Immunological Interest*, (5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)). For example, according to this convention, the immunoglobulin family to which an immunoglobulin sequence of interest belongs is determined based on conservation of variable region polypeptide sequence invariant amino acid residues, to identify a particular numbering system for the immunoglobulin family, and the sequence(s) of interest can then be aligned to assign sequence position numbers to the individual amino acids which comprise such sequence(s). Preferably at least about 70%, more preferably at least about 80%-85% or about 86%-89%, and still more preferably at least about 90%, about 92%, about 94%, about 96%, about 98% or about 99% of the amino acids in a given amino acid sequence of at least about 1000, more preferably about 700-950, more preferably about 350-700, still more preferably about 100-350, still more preferably about 80-100, about 70-80, about 60-70, about 50-60, about 40-50 or about 30-40 consecutive amino acids of a sequence, are identical to the amino acids located at corresponding positions in a reference sequence such as those disclosed by Kabat (1991) or in a similar compendium of related immunoglobulin sequences, such as may be generated from public databases (*e.g.*, Genbank, SwissProt, *etc.*) using sequence alignment tools such as, for example, those described above. In certain preferred embodiments, an immunoglobulin sequence of interest or a region, portion, derivative or fragment thereof is greater than about 95% identical to a corresponding reference sequence, and in certain preferred embodiments such a sequence of interest may differ from a corresponding reference at no more than about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid positions.

For example, in certain embodiments the present invention is directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in

pertinent part a human or other species immunoglobulin heavy chain variable region polypeptide comprising a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112 in, for example, SEQ ID NO: __, which comprises, for example, a murine V_H-
5 derived sequence. At a relatively limited number of immunoglobulin V_H sequence positions, for example, including position 11, amino acid conservation is observed in the overwhelming majority of V_H sequences analyzed across mammalian species lines (e.g., Leu11, Val37, Gly44, Leu45, Trp47; Nguyen *et al.*, 1998 *J. Mol. Biol.* 275:413). Various such amino acid residues, and hence their side chains, are located at the surface of the
10 variable domain (V_H). They may contact residues of the C_H1 (e.g., Leu11) and the V_L domains (e.g., Val37, Gly44, Leu45, and Trp47) and may, in the absence of light chains, contribute to stability and solubility of the protein (see, e.g., Chothia *et al.*, 1985 *J. Mol. Biol.* 186:651; Muyldermans *et al.*, 1994 *Prot. Engineer.* 7:1129; Desmyter *et al.*, 1996 *Nat. Struct. Biol.* 3:803; Davies *et al.*, 1994 *FEBS Lett.* 339:285). In certain embodiments, for
15 example, the present invention is also directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part a human immunoglobulin light chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 12, 80,
20 81, 82, 83, 105, 106, 107 and 108. In still other certain embodiments, for example, the present invention is directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part (1) a human immunoglobulin heavy chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising, consisting essentially of, or consisting of, said heavy
25 chain sequence having a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, and (2) a human immunoglobulin light chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising, consisting essentially of, or consisting of, said light chain sequence having a mutation,

alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108.

As another example, by reference to immunoglobulin sequence compendia and databases such as those cited above, for example, the relatedness of two or more immunoglobulin sequences to each other can readily and without undue experimentation be established in a manner that permits identification of the animal species of origin, the class and subclass (*e.g.*, isotype) of a particular immunoglobulin or immunoglobulin region polypeptide sequence. Any immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences or other native or engineered V region-derived sequences or the like, may be used as a binding region or binding domain. Engineered sequences includes immunoglobulin sequences from any species, preferably human or mouse, for example, that include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112 in a heavy chain variable region sequence or an scFv, and/or a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence or an scFv.

Various preferred embodiments include, for example, native or engineered immunoglobulin V region polypeptide sequences derived, for example, from antibodies including monoclonal antibodies such as murine or other rodent antibodies, or antibodies or monoclonal antibodies derived from other sources such as goat, rabbit, equine, bovine, camelid or other species, including transgenic animals, and also including human or humanized antibodies or monoclonal antibodies. Non-limiting examples include variable region polypeptide sequences derived from monoclonal antibodies such as those referenced herein and/or described in greater detail in the Examples below, for instance, CD20-binding or specific murine monoclonal antibodies (*e.g.*, 2H7), monoclonal antibody L6 (specific for a carbohydrate-defined epitope and available from American Type Culture Collection, Manassas, VA, as hybridoma HB8677), and monoclonal antibodies that bind to or are specific for CD28 (*e.g.*, monoclonal antibody 2E12), CD40, CD80, CD137 (*e.g.*,

monoclonal antibody 5B9 or monoclonal antibody 1D8 which recognizes the murine homologue of CD137, 41BB) and CD152 (CTLA-4).

Other binding regions, including binding domain polypeptides, may comprise any protein or portion thereof that retains the ability to bind or specifically bind to an antigen as provided herein, including non-immunoglobulins. Accordingly the invention contemplates constructs, including fusion proteins, comprising binding region or binding domain polypeptides that are derived from polypeptide ligands such as hormones, cytokines, chemokines, and the like; cell surface or soluble receptors for such polypeptide ligands; lectins; intercellular adhesion receptors such as specific leukocyte integrins, selectins, immunoglobulin gene superfamily members, intercellular adhesion molecules (ICAM-1, -2, -3) and the like; histocompatibility antigens; *etc.*

Examples of cell surface receptors useful in the preparation of, or as, binding regions, or that may provide a binding domain polypeptide, and that may also be selected as a target molecule or antigen to which a construct, including for example, a binding domain-Ig fusion protein of the present invention desirably binds, include the following, or the like: HER1 (*e.g.*, GenBank Accession Nos. U48722, SEG_HEGFREXS, KO3193), HER2 (Yoshino *et al.*, 1994 *J. Immunol.* 152:2393; Disis *et al.*, 1994 *Canc. Res.* 54:16; see also, *e.g.*, GenBank Acc. Nos. X03363, M17730, SEG_HUMHER20), HER3 (*e.g.*, GenBank Acc. Nos. U29339, M34309), HER4 (Plowman *et al.*, 1993 *Nature* 366:473; see also *e.g.*, GenBank Acc. Nos. L07868, T64105), epidermal growth factor receptor (EGFR) (*e.g.*, GenBank Acc. Nos. U48722, SEG_HEGFREXS, KO3193), vascular endothelial cell growth factor (*e.g.*, GenBank No. M32977), vascular endothelial cell growth factor receptor (*e.g.*, GenBank Acc. Nos. AF022375, 1680143, U48801, X62568), insulin-like growth factor-I (*e.g.*, GenBank Acc. Nos. X00173, X56774, X56773, X06043, *see also* European Patent No. GB 2241703), insulin-like growth factor-II (*e.g.*, GenBank Acc. Nos. X03562, X00910, SEG_HUMGFIA, SEG_HUMGFI2, M17863, M17862), transferrin receptor (Trowbridge and Omary, 1981 *Proc. Nat. Acad. USA* 78:3039; see also *e.g.*, GenBank Acc. Nos. X01060, M11507), estrogen receptor (*e.g.*, GenBank Acc. Nos. M38651, X03635, X99101, U47678, M12674), progesterone receptor (*e.g.*, GenBank Acc. Nos. X51730, X69068, M15716), follicle stimulating hormone receptor (FSH-R) (*e.g.*, GenBank Acc. Nos. Z34260, M65085),

retinoic acid receptor (e.g., GenBank Acc. Nos. L12060, M60909, X77664, X57280, X07282, X06538), MUC-1 (Barnes *et al.*, 1989 *Proc. Nat. Acad. Sci. USA* **86**:7159; see also e.g., GenBank Acc. Nos. SEG_MUSMUCIO, M65132, M64928) NY-ESO-1 (e.g., GenBank Acc. Nos. AJ003149, U87459), NA 17-A (e.g., European Patent No. WO 96/40039), Melan-A/MART-1 (Kawakami *et al.*, 1994 *Proc. Nat. Acad. Sci. USA* **91**:3515; see also e.g., GenBank Acc. Nos. U06654, U06452), tyrosinase (Topalian *et al.*, 1994 *Proc. Nat. Acad. Sci. USA* **91**:9461; see also e.g., GenBank Acc. Nos. M26729, SEG_HUMTYR0, see also Weber *et al.*, *J. Clin. Invest* (1998) **102**:1258), Gp-100 (Kawakami *et al.*, 1994 *Proc. Nat. Acad. Sci. USA* **91**:3515; see also e.g., GenBank Acc. No. S73003, see also European Patent No. EP 668350; Adema *et al.*, 1994 *J. Biol. Chem.* **269**:20126), MAGE (van den Bruggen *et al.*, 1991 *Science* **254**:1643; see also e.g., GenBank Acc. Nos. U93163, AF064589, U66083, D32077, D32076, D32075, U10694, U10693, U10691, U10690, U10689, U10688, U10687, U10686, U10685, L18877, U10340, U10339, L18920, U03735, M77481), BAGE (e.g., GenBank Acc. No. U19180; see also U.S. Patent Nos. 5,683,886 and 5,571,711), GAGE (e.g., GenBank Acc. Nos. AF055475, AF055474, AF055473, U19147, U19146, U19145, U19144, U19143, U19142), any of the CTA class of receptors including in particular HOM-MEL-40 antigen encoded by the SSX2 gene (e.g., GenBank Acc. Nos. X86175, U90842, U90841, X86174), carcinoembryonic antigen (CEA, Gold and Freedman, 1985 *J. Exp. Med.* **121**:439; see also e.g., GenBank Acc. Nos. SEG_HUMCEA, M59710, M59255, M29540), and PyLT (e.g., GenBank Acc. Nos. J02289, J02038).

Additional cell surface receptors that may be sources of binding region or binding domain polypeptides or portions thereof, or that may be targets, including target antigens, include the following, or the like: CD2 (e.g., GenBank Acc. Nos. Y00023, SEG_HUMCD2, M16336, M16445, SEG_MUSCD2, M14362), 4-1BB (CDw137, Kwon *et al.*, 1989 *Proc. Nat. Acad. Sci. USA* **86**:1963, 4-1BB ligand (Goodwin *et al.*, 1993 *Eur. J. Immunol.* **23**:2361; Melero *et al.*, 1998 *Eur. J. Immunol.* **3**:116), CD5 (e.g., GenBank Acc. Nos. X78985, X89405), CD10 (e.g., GenBank Acc. Nos. M81591, X76732) CD27 (e.g., GenBank Acc. Nos. M63928, L24495, L08096), CD28 (June *et al.*, 1990 *Immunol. Today* **11**:211; see also, e.g., GenBank Acc. Nos. J02988, SEG_HUMCD28, M34563), CD152/CTLA-4 (e.g., GenBank Acc. Nos. L15006, X05719, SEG_HUMIGCTL), CD40

(e.g., GenBank Acc. Nos. M83312, SEG_MUSC040A0, Y10507, X67878, X96710, U15637, L07414), interferon- γ (IFN- γ ; see, e.g., Farrar *et al.* 1993 *Ann. Rev. Immunol.* **11**:571 and references cited therein, Gray *et al.* 1982 *Nature* **295**:503, Rinderknecht *et al.* 1984 *J. Biol. Chem.* **259**:6790, DeGrado *et al.* 1982 *Nature* **300**:379), interleukin-4 (IL-4; see, e.g., 53rd Forum in Immunology, 1993 *Research in Immunol.* **144**:553-643; Banchereau *et al.*, 1994 in *The Cytokine Handbook*, 2nd ed., A. Thomson, ed., Academic Press, NY, p. 99; Keegan *et al.*, 1994 *J Leukocyt. Biol.* **55**:272, and references cited therein), interleukin-17 (IL-17) (e.g., GenBank Acc. Nos. U32659, U43088) and interleukin-17 receptor (IL-17R) (e.g., GenBank Acc. Nos. U31993, U58917). Notwithstanding the foregoing, the present invention expressly does not encompass any immunoglobulin fusion protein that is disclosed in U.S. 5,807,734, or U.S. 5,795,572.

Additional cell surface receptors that may be sources of binding region or binding domain polypeptides or portions thereof, or that may serve as targets including target antigens or binding sites include the following, or the like: CD59 (e.g., GenBank Acc. Nos. SEG_HUMCD590, M95708, M34671), CD48 (e.g., GenBank Acc. Nos. M59904), CD58/LFA-3 (e.g., GenBank Acc. No. A25933, Y00636, E12817; see also JP 1997075090-A), CD72 (e.g., GenBank Acc. Nos. AA311036, S40777, L35772), CD70 (e.g., GenBank Acc. Nos. Y13636, S69339), CD80/B7.1 (Freeman *et al.*, 1989 *J. Immunol.* **43**:2714; Freeman *et al.*, 1991 *J. Exp. Med.* **174**:625; see also e.g., GenBank Acc. Nos. U33208, I683379), CD86/B7.2 (Freeman *et al.*, 1993 *J. Exp. Med.* **178**:2185, Boriello *et al.*, 1995 *J. Immunol.* **155**:5490; see also, e.g., GenBank Acc. Nos. AF099105, SEG_MMB72G, U39466, U04343, SEG_HSB725, L25606, L25259), B7-H1/B7-DC (e.g., Genbank Acc. Nos. NM_014143, AF177937, AF317088; Dong *et al.*, 2002 *Nat. Med.* Jun 24 [epub ahead of print], PMID 12091876; Tseng *et al.*, 2001 *J. Exp. Med.* **193**:839; Tamura *et al.*, 2001 *Blood* **97**:1809; Dong *et al.*, 1999 *Nat. Med.* **5**:1365), CD40 ligand (e.g., GenBank Acc. Nos. SEG_HUMCD40L, X67878, X65453, L07414), IL-17 (e.g., GenBank Acc. Nos. U32659, U43088), CD43 (e.g., GenBank Acc. Nos. X52075, J04536), ICOS (e.g., Genbank Acc. No. AH011568), CD3 (e.g., Genbank Acc. Nos. NM_000073 (gamma subunit), NM_000733 (epsilon subunit), X73617 (delta subunit)), CD4 (e.g., Genbank Acc. No. NM_000616), CD25 (e.g., Genbank Acc. No. NM_000417), CD8 (e.g., Genbank Acc. No. M12828),

CD11b (*e.g.*, Genbank Acc. No. J03925), CD14 (*e.g.*, Genbank Acc. No. XM_039364), CD56 (*e.g.*, Genbank Acc. No. U63041), CD69 (*e.g.*, Genbank Acc. No. NM_001781) and VLA-4 ($\alpha_4\beta_7$) (*e.g.*, GenBank Acc. Nos. L12002, X16983, L20788, U97031, L24913, M68892, M95632). The following cell surface receptors are typically associated with B cells: CD19 (*e.g.*, GenBank Acc. Nos. SEG_HUMCD19W0, M84371, SEG_MUSCD19W, M62542), CD20 (*e.g.*, GenBank Acc. Nos. SEG_HUMCD20, M62541), CD22 (*e.g.*, GenBank Acc. Nos. I680629, Y10210, X59350, U62631, X52782, L16928), CD30 (*e.g.*, Genbank Acc. Nos. M83554, D86042), CD153 (CD30 ligand, *e.g.*, GenBank Acc. Nos. L09753, M83554), CD37 (*e.g.*, GenBank Acc. Nos. SEG_MMCD37X, X14046, X53517), CD50 (ICAM-3, *e.g.*, GenBank Acc. No. NM_002162), CD106 (VCAM-1) (*e.g.*, GenBank Acc. Nos. X53051, X67783, SEG_MMVCAM1C, *see also* U.S. Patent No. 5,596,090), CD54 (ICAM-1) (*e.g.*, GenBank Acc. Nos. X84737, S82847, X06990, J03132, SEG_MUSICAM0), interleukin-12 (*see, e.g.*, Reiter *et al*, 1993 *Crit. Rev. Immunol.* 13:1, and references cited therein), CD134 (OX40, *e.g.*, GenBank Acc. No. AJ277151), CD137 (41BB, *e.g.*, GenBank Acc. No. L12964, NM_001561), CD83 (*e.g.*, GenBank Acc. Nos. AF001036, AL021918), DEC-205 (*e.g.*, GenBank Acc. Nos. AF011333, U19271).

Constructs, including binding domain-immunoglobulin fusion proteins, of the present invention comprise, for example, a binding domain, such as a binding domain polypeptide that, according to certain particularly preferred embodiments, is capable of binding or specifically binding at least one target, for example, a target antigen or other binding site that is present on an immune effector cell. According to non-limiting theory, such constructs, including for example binding domain- immunoglobulin fusion proteins, may advantageously recruit desired immune effector cell function(s) in a therapeutic context, where it is well known that immune effector cells having different specialized immune functions can be identified or distinguished from one another on the basis of their differential expression of a wide variety of cell surface antigens, including many of the antigens described herein to which constructs of the invention including binding domain polypeptides can specifically bind. As noted herein, immune effector cells include any cell that is capable of directly mediating an activity which is a component of immune system

function, including cells having such capability naturally or as a result of genetic engineering.

In certain embodiments an immune effector cell comprises a cell surface receptor for an immunoglobulin or other peptide binding molecule, such as a receptor for an immunoglobulin constant region and including the class of receptors commonly referred to as "Fc receptors" ("FcR"s). A number of FcRs have been structurally and/or functionally characterized and are well known in the art, including FcR having specific abilities to interact with a restricted subset of immunoglobulin heavy chain isotypes, or that interact with Fc domains with varying affinities, and/or which may be expressed on restricted subsets of immune effector cells under certain conditions (*e.g.*, Kijimoto-Ochichai *et al.*, 2002 *Cell Mol. Life Sci.* **59**:648; Davis *et al.*, 2002 *Curr. Top. Microbiol. Immunol.* **266**:85; Pawankar, 2001 *Curr. Opin. Allerg. Clin. Immunol.* **1**:3; Radaev *et al.*, 2002 *Mol. Immunol.* **38**:1073; Wurzburg *et al.*, 2002 *Mol. Immunol.* **38**:1063; Sulica *et al.*, 2001 *Int. Rev. Immunol.* **20**:371; Underhill *et al.*, 2002 *Ann. Rev. Immunol.* **20**:825; Coggeshall, 2002 *Curr. Dir. Autoimm.* **5**:1; Mimura *et al.*, 2001 *Adv. Exp. Med. Biol.* **495**:49; Baumann *et al.*, 2001 *Adv. Exp. Med. Biol.* **495**:219; Santoso *et al.*, 2001 *Ital. Heart J.* **2**:811; Novak *et al.*, 2001 *Curr. Opin. Immunol.* **13**:721; Fossati *et al.*, 2001 *Eur. J. Clin. Invest.* **31**:821).

Cells that are capable of mediating ADCC are preferred examples of immune effector cells according to the present invention. Other preferred examples include Natural Killer cells, tumor-infiltrating T lymphocytes (TILs), cytotoxic T lymphocytes, and granulocytic cells such as cells that comprise allergic response mechanisms. Immune effector cells thus include, but are not limited to, cells of hematopoietic origins including cells at various stages of differentiation within myeloid and lymphoid lineages and which may (but need not) express one or more types of functional cell surface FcR, such as T lymphocytes, B lymphocytes, NK cells, monocytes, macrophages, dendritic cells, neutrophils, basophils, eosinophils, mast cells, platelets, erythrocytes, and precursors, progenitors (*e.g.*, hematopoietic stem cells), as well as quiescent, activated, and mature forms of such cells. Other immune effector cells may include cells of non-hematopoietic origin that are capable of mediating immune functions, for example, endothelial cells, keratinocytes, fibroblasts, osteoclasts, epithelial cells, and other cells. Immune effector cells

may also include cells that mediate cytotoxic or cytostatic events, or endocytic, phagocytic, or pinocytotic events, or that effect induction of apoptosis, or that effect microbial immunity or neutralization of microbial infection, or cells that mediate allergic, inflammatory, hypersensitivity and/or autoimmune reactions.

5 Allergic response mechanisms are well known in the art and include an antigen (*e.g.*, allergen)-specific component such as an immunoglobulin (*e.g.*, IgE), as well as the cells and mediators which comprise sequelae to allergen-immunoglobulin (*e.g.*, IgE) encounters (*e.g.*, Ott *et al.*, 2000 *J. Allerg. Clin. Immunol.* **106**:429; Barnes, 2000 *J. Allerg. Clin. Immunol.* **106**:5; Togias, 2000 *J. Allerg. Clin. Immunol.* **105**:S599; Akdis *et al.*, 2000
10 *Int. Arch. Allerg. Immunol.* **121**:261; Beach, 2000 *Occup. Med.* **15**:455). Particularly with regard to constructs, including binding domain-immunoglobulin fusion proteins, of the present invention that interact with FcR, certain embodiments of the present invention contemplate constructs including fusion proteins that comprise one or more IgE-derived domains including, for example, those that are capable of inducing an allergic response
15 mechanism that comprises IgE-specific FcR, or portions thereof, which IgE-specific FcRs include those noted above and described or identified in the cited articles. Without wishing to be bound by particular theory or mechanism, and as disclosed herein, constructs, including fusion proteins, of the present invention may comprise portions of IgE heavy chain Fc domain polypeptides, for example, native or engineered IgE CH3 and CH4
20 domains, whether provided or expressed as cell surface proteins (*e.g.*, with a plasma membrane anchor domain) or as soluble or otherwise not cell-bound proteins (*e.g.*, without a plasma membrane anchor domain). Further according to non-limiting theory, recruitment and induction of an allergic response mechanism (*e.g.*, an FcR-epsilon expressing immune effector cell) may proceed as the result of either or both of the presence of an IgE Fc domain
25 or portion thereof as described herein (*e.g.*, one that is capable of triggering an allergic mechanism by FcR crosslinking) and the presence of a target such as a antigen to which the binding region, for example a binding domain, binds or specifically binds. The present invention therefore exploits induction of allergic response mechanisms in heretofore unappreciated contexts, such as treatment of a malignant condition or a B cell disorder,
30 including those described or referenced herein.

An immunoglobulin hinge region polypeptide includes any hinge peptide or polypeptide that occurs naturally, as an artificial peptide or as the result of genetic engineering and that is situated, for example, in an immunoglobulin heavy chain polypeptide between the amino acid residues responsible for forming intrachain immunoglobulin-domain disulfide bonds in CH1 and CH2 regions. Hinge region polypeptides for use in the present invention may also include a mutated or otherwise altered hinge region polypeptide. Accordingly, for example, an immunoglobulin hinge region polypeptide may be derived from, or may be a portion or fragment of (*i.e.*, one or more amino acids in peptide linkage, typically about 15-115 amino acids, preferably about 95-110, about 80-94, about 60-80, or about 5-65 amino acids, preferably about 10-50, more preferably about 15-35, still more preferably about 18-32, still more preferably about 20-30, still more preferably about 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acids) an immunoglobulin polypeptide chain region classically regarded as having hinge function, including those described herein, but a hinge region polypeptide for use in the instant invention need not be so restricted and may include one or more amino acids situated (according to structural criteria for assigning a particular residue to a particular domain that may vary, as known in the art) in an adjoining immunoglobulin domain such as a CH1 domain and/or a CH2 domain in the cases of IgG, IgA and IgD (or in an adjoining immunoglobulin domain such as a CH1 domain and/or a CH3 domain in the case of IgE), or in the case of certain artificially engineered immunoglobulin constructs, an immunoglobulin variable region domain.

Wild-type immunoglobulin hinge region polypeptides include any known or later-discovered naturally occurring hinge region that is located between the constant region domains, CH1 and CH2, of an immunoglobulin, for example, a human immunoglobulin (or between the CH1 and CH3 regions of certain types of immunoglobulins, such as IgE),. For use in constructing one type of connecting region, the wild-type immunoglobulin hinge region polypeptide is preferably a human immunoglobulin hinge region polypeptide, preferably comprising a hinge region from a human IgG, IgA, or IgD immunoglobulin (or the CH2 region of an IgE immunoglobulin), and more preferably, for example, a hinge region polypeptide from a wild-type or mutated human IgG1 isotype as described herein.

As is known to the art, despite the tremendous overall diversity in immunoglobulin amino acid sequences, immunoglobulin primary structure exhibits a high degree of sequence conservation in particular portions of immunoglobulin polypeptide chains, notably with regard to the occurrence of cysteine residues which, by virtue of their
5 sulfhydryl groups, offer the potential for disulfide bond formation with other available sulfhydryl groups. Accordingly, in the context of the present invention wild-type immunoglobulin hinge region polypeptides for use as connecting regions include those that feature one or more highly conserved (*e.g.*, prevalent in a population in a statistically significant manner) cysteine residues, and in certain preferred embodiments a connecting
10 region may comprise, or consist essentially of, or consist of, a mutated hinge region polypeptide may be selected that contains less than the number of naturally-occurring cysteines, for example, zero or one or two cysteine residue(s) in the case of IgG1 and IgG4 hinge regions, and that is derived or constructed from (or using) such a wild-type hinge region sequence.

15 In certain preferred embodiments wherein the connecting region is a hinge region polypeptide and the hinge region polypeptide is a mutated, engineered or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that is derived or constructed from (or using) a wild-type hinge region sequence, it is noted that the wild-type human IgG1 hinge region polypeptide sequence comprises three non-adjacent cysteine residues, referred
20 to as a first cysteine of the wild-type hinge region, a second cysteine of the wild-type hinge region and a third cysteine of the wild-type hinge region, respectively, proceeding along the hinge region sequence from the polypeptide N-terminus toward the C-terminus. This can be referred to herein as a "CCC" hinge (or a "WTH", *i.e.*, a wild-type hinge). Examples of mutated or engineered hinge regions include those with no cysteines, which may be referred
25 to herein as an "XXX" hinge (or, for example, as "MH-XXX," referring to a mutant or engineered hinge with three amino acids or other molecules in place of naturally occurring cysteines, such as, for example, "MH-SSS", which refers to a mutant hinge with three serine residues in place of the naturally occurring cysteine residues. It will be understood that the term "mutant" refers only to the fact that a different molecule or molecules is present, or no
30 molecule, at the position of a naturally occurring residue and does not refer to any particular

method by which such substitution, alteration, or deletion has been carried out. Accordingly, in certain embodiments of the present invention, the connecting region may be a hinge region polypeptide and the hinge region polypeptide is a mutated human IgG1 immunoglobulin hinge region polypeptide that contains two cysteine residues and in which the first cysteine of the wild-type hinge region has not changed or deleted, for example. This can be referred to as a "MH-CXX" hinge, for example, a "MH-CSC" hinge, in which case the cysteine residue has been replaced with a serine residue. In certain other embodiments of the present invention the mutated human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue and include, for example, a "MH-CSS" hinge or a "MH-SSC" hinge or a "MH-CSC" hinge, and in certain other embodiments the mutated human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues such as, for example, a "MH-SSS" hinge.

The constructs, including binding domain-immunoglobulin fusion proteins, of the present invention expressly do not contemplate any fusion protein that is disclosed in U.S. Patent No. 5,892,019. U.S. Patent No. 5,892,019 refers to a human IgG1 hinge region in which the first IgG1 hinge region cysteine residue has been changed or deleted, but retains both of the second and third IgG1 hinge region cysteine residues that correspond to the second and third cysteines of the wild-type IgG1 hinge region sequence. The patent states that the first cysteine residue of the wild-type IgG1 hinge region is replaced to prevent interference by the first cysteine residue with proper assembly of the polypeptide described therein into a dimer. The patent requires that the second and third cysteines of the IgG1 hinge region be retained to provide interchain disulfide linkage between two heavy chain constant regions to promote dimer formation so that the molecule contains has effector function such as the ability to mediate ADCC.

By contrast and as described herein, the constructs, including the binding domain-immunoglobulin fusion proteins, of the present invention, various of which are capable of ADCC, CDC and/or complement fixation, for example, are not so limited and may comprise, in pertinent part, for example, (i) a wild-type immunoglobulin hinge region polypeptide, such as a wild-type human immunoglobulin hinge region polypeptide, for example, a human IgG1 immunoglobulin hinge region polypeptide, (ii) a mutated or

otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that, for example, is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated or deleted, (iii) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that, for example, is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue, or (iv) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered (for example, by amino acid change or deletion) human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues. The present invention offers unexpected advantages associated with retention by the constructs, including the fusion proteins, described herein of the ability to mediate ADCC and/or CDC and/or complement fixation notwithstanding that the ability to dimerize via IgG1 hinge region interchain disulfide bonds is ablated or compromised by the removal or replacement of one, two or three hinge region cysteine residues, and even in constructs where the first cysteine of an IgG1 hinge region, for example, is not mutated or otherwise altered or deleted.

A connecting region may comprise a mutated or otherwise altered immunoglobulin hinge region polypeptide, which itself may comprise a hinge region that

has its origin in an immunoglobulin of a species, of an immunoglobulin isotype or class, or of an immunoglobulin subclass that is different from that of the tail region, for example, a tail region comprising, or consisting essentially of, or consisting of, CH2 and CH3 domains (or IgE CH3 and CH4 domains). For instance, in certain embodiments of the invention, a construct, for example, a binding domain-immunoglobulin fusion protein, may comprise a binding region such as a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide comprising, or consisting essentially of, or consisting of, a wild-type human IgA hinge region polypeptide, or a mutated or otherwise altered human IgA hinge region polypeptide that contains zero or only one or more cysteine residues (but less than the wild-type number of cysteines), as described herein, or a wild-type human IgG hinge, such as an IgG1 hinge, region polypeptide, or a wild-type human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide, or a mutated or otherwise altered human IgG hinge, such as an IgG1 hinge, region polypeptide that is or has been mutated or otherwise altered to contain zero, one or two cysteine residues wherein the first cysteine of the wild-type hinge region is not mutated or altered or deleted, as also described herein. Such a hinge region polypeptide may be fused or otherwise connected to, for example, a tail region comprising, or consisting essentially of, or consisting of, an immunoglobulin heavy chain CH2 region polypeptide from a different Ig isotype or class, for example an IgA or an IgD or an IgG subclass (or a CH3 region from an IgE subclass), which in certain preferred embodiments will be the IgG1 or IgA or IgE subclass and in certain other preferred embodiments may be any one of the IgG2, IgG3 or IgG4 subclasses.

For example, and as described in greater detail herein, in certain embodiments of the present invention a connecting region may be selected to be an immunoglobulin hinge region polypeptide, which is or has been derived from a wild-type human IgA hinge region that naturally comprises three cysteines, where the selected hinge region polypeptide is truncated or otherwise altered or substituted relative to the complete and/or naturally-occurring hinge region such that only one or two of the cysteine residues remain (*e.g.*, SEQ ID NOS:35-36). Similarly, in certain other embodiments of the invention, the construct may be binding domain-immunoglobulin fusion protein comprising a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region

polypeptide comprising a mutated or otherwise altered hinge region polypeptide in which the number of cysteine residues is reduced by amino acid substitution or deletion, for example a mutated or otherwise altered IgG1 hinge region containing zero, one or two cysteine residues as described herein, or an IgD hinge region containing zero cysteine residues.

A mutated or otherwise altered hinge region polypeptide may thus be derived or constructed from (or using) a wild-type immunoglobulin hinge region that contains one or more cysteine residues. In certain embodiments, a mutated or otherwise altered hinge region polypeptide may contain zero or only one cysteine residue, wherein the mutated or otherwise altered hinge region polypeptide is or has been derived from a wild type immunoglobulin hinge region that contains, respectively, one or more or two or more cysteine residues. In the mutated or otherwise altered hinge region polypeptide, the cysteine residues of the wild-type immunoglobulin hinge region are preferably deleted or substituted with amino acids that are incapable of forming a disulfide bond. In one embodiment of the invention, a mutated or otherwise altered hinge region polypeptide is or has been derived from a human IgG wild-type hinge region polypeptide, which may include any of the four human IgG isotype subclasses, IgG1, IgG2, IgG3 or IgG4. In certain preferred embodiments, the mutated or otherwise altered hinge region polypeptide is or has been derived from (or using) a human IgA or IgD wild-type hinge region polypeptide. By way of example, a mutated or otherwise altered hinge region polypeptide that is or has been derived from a human IgG1 or IgA wild-type hinge region polypeptide may comprise mutations, alterations, or deletions at two of the three cysteine residues in the wild-type immunoglobulin hinge region, or mutations, alterations, or deletions at all three cysteine residues.

The cysteine residues that are present in a wild-type immunoglobulin hinge region and that are removed or altered by mutagenesis or any other techniques according to particularly preferred embodiments of the present invention include cysteine residues that form, or that are capable of forming, interchain disulfide bonds. Without wishing to be bound by particular theory or mechanism of action, the present invention contemplates that mutation, deletion, or other alteration of such hinge region cysteine residues, which are

believed to be involved in formation of interchain disulfide bridges, reduces the ability of the subject invention binding domain-immunoglobulin fusion protein to dimerize (or form higher oligomers) via interchain disulfide bond formation, while surprisingly not ablating or undesireably compromising the ability of a fusion protein or other construct to promote ADCC, and/or CDC and/or to fix complement. In particular, the Fc receptors that mediate ADCC (*e.g.*, FcRIII, CD16) exhibit low affinity for immunoglobulin Fc domains, supporting the idea that functional binding of Fc to FcR requires avidity stabilization of the Fc-FcR complex by virtue of the dimeric structure of heavy chains in a conventional antibody, and/or FcR aggregation and cross-linking by a conventional antibody Fc structure.

Sonderman *et al.*, 2000 *Nature* **406**:267; Radaev *et al.*, 2001 *J. Biol. Chem.* **276**:16469; Radaev *et al.*, 2001 *J. Biol. Chem.* **276**:16478; Koolwijk *et al.*, 1989 *J. Immunol.* **143**:1656; Kato *et al.*, 2000 *Immunol. Today* **21**:310. Hence, the constructs, including for example binding domain-immunoglobulin fusion proteins, of the present invention provide the advantages associated with single-chain constructs including single-chain immunoglobulin fusion proteins while also unexpectedly retaining one or more immunological activities. Similarly, the ability to fix complement is typically associated with immunoglobulins that are dimeric with respect to heavy chain constant regions such as those that comprise Fc, while various constructs, including binding domain-immunoglobulin fusion proteins, of the present invention, which may, due to the replacement or deletion of hinge region cysteine residues or due to other structural modifications as described herein, for example, have compromised or ablated abilities to form interchain disulfide bonds, exhibit the unexpected ability to fix complement. Additionally, according to certain embodiments of the present invention wherein a construct, including, for example, a binding domain-immunoglobulin fusion protein, may comprise a connecting region and tail region comprising, or consisting essentially of, or consisting of, one or more of a human IgE hinge-acting region, *i.e.*, a IgE CH2 region polypeptide, a human IgE CH3 constant region polypeptide, and a human IgE CH4 constant region polypeptide, the invention constructs including fusion proteins unexpectedly retain the immunological activity of mediating ADCC and/or of inducing an allergic response mechanism.

Selection of an immunoglobulin hinge region polypeptide as a connecting region according to certain embodiments of the subject invention constructs, such as binding domain-immunoglobulin fusion proteins, may relate to the use of an “alternative hinge region” polypeptide sequence, which includes a polypeptide sequence that is not necessarily derived from any immunoglobulin hinge region sequence *per se*. Instead, an alternative hinge region refers to a hinge region polypeptide that comprises an amino acid sequence, or other molecular sequence, of at least about ten consecutive amino acids or molecules, and in certain embodiments at least about 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30, 31-50, 51-60, 71-80, 81-90, or 91-110 amino acids or molecules that is present in a sequence selected from any one of SEQ ID NOS: __-__, for example a polypeptide sequence that is or has been derived from a region located between intrachain disulfide-generated immunoglobulin-like loop domains of immunoglobulin gene superfamily members such as CD2 (*e.g.*, Genbank Acc. No. NM_001767), CD4 (*e.g.*, Genbank Acc. No. NM_000616), CD5 (*e.g.*, Genbank Acc. No. BC027901), CD6 (*e.g.*, Genbank Acc. No. NM_006725), CD7 (*e.g.*, Genbank Acc. Nos. XM_046782, BC009293, NM_006137) or CD8 (*e.g.*, Genbank Acc. No. M12828), or other Ig superfamily members. By way of non-limiting example, an alternative hinge region used as a connecting region, for example, may provide a glycosylation site as provided herein, or may provide a human gene-derived polypeptide sequence for purposes of enhancing the degree of “humanization” of a fusion protein, or may comprise, or consist essentially of, or consist of, an amino acid sequence that eliminates or reduces the ability of a construct of the invention, such as a fusion protein, to form multimers or oligomers or aggregates or the like. Certain alternative hinge region polypeptide sequences, including those described herein, may be derived or constructed from (or using) the polypeptide sequences of immunoglobulin gene superfamily members that are not actual immunoglobulins *per se*. For instance and according to non-limiting theory, certain polypeptide sequences that are situated between intrachain disulfide-generated immunoglobulin loop domain of immunoglobulin gene super-family member proteins may be used in whole or in part as alternative hinge region polypeptides as provided herein, or may be further modified for such use.

As noted above, the constructs of the invention, including binding domain-immunoglobulin fusion proteins, are believed, according to non-limiting theory, to be compromised in their ability to dimerize via interchain disulfide bond formation, and further according to theory, this property is a consequence, in whole or in part, of a reduction in the number of cysteine residues that are present in an immunoglobulin hinge region polypeptide selected for inclusion in the construction of the construct, such as a fusion protein construct. Determination of the relative ability of a polypeptide to dimerize is well within the knowledge of the relevant art, where any of a number of established methodologies may be applied to detect protein dimerization (see, *e.g.*, Scopes, *Protein Purification: Principles and Practice*, 1987 Springer-Verlag, New York). For example, biochemical separation techniques for resolving proteins on the basis of molecular size (*e.g.*, gel electrophoresis, gel filtration chromatography, analytical ultracentrifugation, *etc.*), and/or comparison of protein physicochemical properties before and after introduction of sulfhydryl-active (*e.g.*, iodoacetamide, N-ethylmaleimide) or disulfide-reducing (*e.g.*, 2-mercaptoethanol, dithiothreitol) agents, or other equivalent methodologies, may all be employed for determining a degree of polypeptide dimerization or oligomerization, and for determining possible contribution of disulfide bonds to such potential quaternary structure. In certain embodiments, the invention relates to a construct, for example a binding domain-immunoglobulin fusion protein, that exhibits a reduced (*i.e.*, in a statistically significant manner relative to an appropriate IgG-derived control, for example) ability to dimerize, relative to a wild-type human immunoglobulin G hinge region polypeptide as provided herein. Those familiar with the art will be able readily to determine whether a particular fusion protein displays such reduced ability to dimerize.

Compositions and methods for preparation of immunoglobulin fusion proteins, for example, are well known in the art. See, *e.g.*, U.S. Patent No. 5,892,019, which reports recombinant proteins that are the products of a single encoding polynucleotide but which are not constructs, including binding domain-immunoglobulin fusion proteins, according to the present invention.

For a construct, for example, an immunoglobulin fusion protein of the invention which is intended for use in humans, any included Ig constant regions will

typically be of human sequence origin, or humanized, to minimize a potential anti-human immune response and to provide appropriate and/or desired effector functions. Manipulation of sequences encoding antibody constant regions is referenced in the PCT publication of Morrison and Oi, WO 89/07142. In particularly preferred embodiments, a tail region is prepared from an immunoglobulin heavy chain constant region in which the CH1 domain is or has been deleted (the CH1 and CH2 regions in the case of IgE) and the carboxyl end of the binding domain, or where the binding domain comprises two immunoglobulin variable region polypeptides, the second (*i.e.*, more proximal to the C-terminus) variable region is joined to the amino terminus of CH2 through one or more connecting regions, such as a hinge or altered region. A schematic diagram depicting the structures of two exemplary binding domain-immunoglobulin fusion proteins is shown in FIG. 11. In particularly preferred embodiments no interchain disulfide bonds are present, and in other embodiments a restricted number of interchain disulfide bonds may be present relative to the number of such bonds that would be present if wild-type hinge region polypeptides were instead present. In other embodiments a construct of the invention, such as for example, a fusion protein, comprises, or consists essentially of, or consists of, a mutated or otherwise altered hinge region polypeptide that exhibits a reduced ability to dimerize, relative to a wild-type human IgG hinge region polypeptide. Thus, an isolated polynucleotide molecule coding for such a single chain construct, such as an immunoglobulin fusion protein, has a binding region, for example, a domain that provides specific or otherwise desired binding affinity and selectivity for a target, such as a target antigen.

The invention also contemplates, for example, in certain embodiments, constructs including binding domain-immunoglobulin fusion proteins that comprise fused or otherwise connected polypeptide sequences or portions thereof derived or prepared from a plurality of genetic sources, for example, according to molecular "domain swapping" paradigms. Those having familiarity with the art will appreciate that selection of such polypeptide sequences for assembly into a construct, such as a binding domain-immunoglobulin fusion protein, for example, may involve determination of appropriate portions of each component polypeptide sequence, for example, based on structural and/or

functional properties of each such sequence (see, e.g., Carayannopoulos *et al.*, 1996 *J. Exp. Med.* **183**:1579; Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988)). The component polypeptide sequences of which the construct, such as a fusion protein, is comprised or prepared may therefore comprise
5 intact or full-length binding domain, immunoglobulin, linker and/or plasma membrane anchor domain polypeptide sequences, or truncated versions or variants thereof such as those provided herein. According to these and related embodiments of the invention, any two or more of the candidate component polypeptides of which the subject invention constructs, for example, fusion proteins, may be comprised will be derived or prepared from
10 independent sources, such as from immunoglobulin sequences of differing allotype, isotype, subclass, class, or species of origin (e.g., xenotype). Thus, as a non-limiting example, a binding domain polypeptide (or its constituent polypeptides such as one or more variable region polypeptides and/or a linker polypeptide), a hinge region polypeptide, immunoglobulin heavy chain CH2 and CH3 constant region polypeptides and optionally an
15 immunoglobulin heavy chain CH4 constant region polypeptide as may be obtained from an IgM or IgE heavy chain, and a plasma membrane anchor domain polypeptide may all be separately obtained from distinct genetic sources and engineered into a chimeric or fusion protein using well known techniques and according to methodologies described herein, for example.

20 Accordingly, a construct of the invention, for example a binding domain-immunoglobulin fusion protein according to certain embodiments of the present invention, may also therefore comprise in pertinent part an immunoglobulin heavy chain CH3 constant region polypeptide that is a wild-type IgA CH3 constant region polypeptide, or alternatively, that is a mutated or otherwise altered or substituted or truncated IgA CH3 constant region
25 polypeptide that is incapable of associating with a J chain, or that will not associate to an undesired degree with a J chain; preferably the IgA CH3 constant region polypeptides used in a tail region portion of a construct are of human origin or are humanized. By way of brief background, IgA molecules are known to be released into secretory fluids by a mechanism that involves association of IgA into disulfide-linked polymers (e.g., dimers) via a J chain
30 polypeptide (e.g., Genbank Acc. Nos. XM_059628, M12378, M12759; Johansen *et al.*, 1999

Eur. J. Immunol. **29**:1701) and interaction of the complex so formed with another protein that acts as a receptor for polymeric immunoglobulin, and which is known as transmembrane secretory component (SC; Johansen *et al.*, 2000 *Sc. J. Immunol.* **52**:240; see also, *e.g.*, Sorensen *et al.*, 2000 *Int. Immunol.* **12**:19; Yoo *et al.*, 1999 *J. Biol. Chem.* **274**:33771; Yoo *et al.*, 2002 *J. Immunol. Meth.* **261**:1; Cortesy, 2002 *Trends Biotechnol.* **20**:65; Symersky *et al.*, 2000 *Mol. Immunol.* **37**:133; Crottet *et al.*, 1999 *Biochem. J.* **341**:299). Interchain disulfide bond formation between IgA Fc domains and J chain is mediated through a penultimate cysteine residue in an 18-amino acid C-terminal extension that forms part of the IgA heavy chain constant region CH3 domain polypeptide (Yoo *et al.*, 1999; Sorensen *et al.*, 2000). Certain embodiments of the subject invention constructs, including for example, fusion proteins, therefore contemplate inclusion of the wild-type IgA heavy chain constant region polypeptide sequence, which is capable of associating with J chain. Certain other embodiments of the invention, however, contemplate fusion proteins that comprise a mutated or otherwise altered, substituted, or truncated IgA CH3 constant region polypeptide that is incapable of associating with a J chain. According to such embodiments, for example, two or more residues from the C-terminus of an IgA CH3 constant region polypeptide such as a human IgA CH3 constant region polypeptide may be deleted to yield a truncated CH3 constant region polypeptide as provided herein. In preferred embodiments and as described in greater detail herein, a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain comprises such a C-terminal deletion of either four or 18 amino acids. However, the invention need not be so limited, such that the mutated IgA CH3 constant region polypeptide may comprise a deletion of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,, 21-25, 26-30 or more amino acids, so long as the construct, for example, the fusion protein, is capable of specifically binding an antigen and of at least one immunological activity as provided herein. Alternatively, the invention also contemplates constructs, for example, fusion proteins, having a tail region that comprises a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain by virtue of replacement of the penultimate cysteine, or by chemical modification of that amino acid residue, in a manner that prevents, or inhibits an undesired level of, interchain disulfide bond or multimer formation. Methods

for determining whether a construct, for example a fusion protein, can associate with a J chain will be known to those having familiarity with the art and are described or referenced herein.

As also described herein and according to procedures known in the art, the construct, for example a fusion protein, may further be tested routinely for immunological activity, for instance, in ADCC or CDC assays. As an illustrative example, a construct, for example a fusion protein, according to such an embodiment may comprise a binding domain polypeptide derived or constructed from (or using) a native or engineered human heavy chain variable region polypeptide sequence, a native or engineered human IgA-derived immunoglobulin hinge region polypeptide sequence, a native or engineered human IgG1 immunoglobulin heavy chain CH2 constant region polypeptide sequence, a native or engineered human IgG2 immunoglobulin heavy chain CH3 constant region polypeptide sequence, and optionally a native or engineered human IgE immunoglobulin heavy chain CH4 constant region polypeptide sequence and/or a native or engineered human TNF- α receptor type 1 (TNFR1) plasma membrane anchor domain polypeptide sequence that comprises a cytoplasmic tail polypeptide which is capable of apoptotic signaling or otherwise promoting apoptosis. The invention therefore contemplates these and other embodiments according to the present invention in which two or more polypeptide sequences that are present in a construct, for example a fusion protein, have independent genetic origins.

As noted above, in certain embodiments the construct, for example a binding protein-immunoglobulin fusion protein, comprises at least one native or engineered immunoglobulin variable region polypeptide, which may be a native or engineered light chain or a native or engineered heavy chain variable region polypeptide, and in certain embodiments the fusion protein comprises at least one such native or engineered light chain V-region and one such native or engineered heavy chain V-region and at least one linker peptide that is fused or otherwise connected to to each of the native or engineered V-regions. Construction of such binding domains, for example single chain Fv domains, is known in the art and is described in greater detail in the Examples below, and has been described, for example, in various documents cited herein; selection and assembly of single-chain variable

regions and of linker polypeptides that may be fused or otherwise connected to each of a heavy chain-derived and a light chain-derived V region (*e.g.*, to generate a binding region, such as a binding domain that comprises a single-chain Fv polypeptide) is also known to the art and described herein. See, *e.g.*, U.S. Patent Nos. 5,869,620, 4,704,692, and 4,946,778.

5 In certain embodiments all or a portion or portions of an immunoglobulin sequence that is derived from a non-human source may be “humanized” according to recognized procedures for generating humanized antibodies, *i.e.*, immunoglobulin sequences into which human Ig sequences are introduced to reduce the degree to which a human immune system would perceive such proteins as foreign (see, *e.g.*, U.S. Patent Nos. 5,693,762; 5,585,089; 10 4,816,567; 5,225,539; 5,530,101; and documents cited therein).

Constructs of the invention, including binding domain-immunoglobulin fusion proteins, as described herein may, according to certain embodiments, desirably comprise sites for glycosylation, *e.g.*, covalent attachment of carbohydrate moieties such as, for example, monosaccharides or oligosaccharides. Incorporation of amino acid sequences that provide substrates for polypeptide glycosylation is within the scope of the relevant art, 15 including, for example, the use of genetic engineering or protein engineering methodologies to obtain a polypeptide sequence containing, for example, the classic Asn-X-Ser/Thr site for N-(asparagine)-linked glycosylation, or a sequence containing Ser or Thr residues that are suitable substrates for O-linked glycosylation, or sequences amenable to C-mannosylation, 20 glypiation/ glycosylphosphatidylinositol modification, or phosphoglycation, all of which can be identified according to art-established criteria (*e.g.*, Spiro, 2002 *Glybiology* 12:43R). Without wishing to be bound by any particular theory or mechanism, glycosylated constructs such as fusion proteins having particular amino acid sequences may beneficially possess attributes associated with one or more of improved solubility, enhanced stability in 25 solution, enhanced physiological stability, improved bioavailability including *in vivo* biodistribution, and superior resistance to proteases, all in a statistically significant manner, relative to constructs, including fusion proteins, having the same or highly similar amino acid sequences but lacking glycosyl moieties. In certain preferred embodiments the subject invention constructs, such as fusion protein constructs, may comprise a glycosylation site 30 that is present in a linker as provided herein, and in certain other preferred embodiments the

subject invention construct, for example, a fusion protein, comprises a glycosylation site that is present in a connecting region, such as a hinge region polypeptide sequence as provided herein.

In certain preferred embodiments of the present invention, such as those
5 useful for gene therapy applications or in display systems or assays, such as screening assays (including library display systems and library screening assays), the construct, for example, a binding domain-immunoglobulin fusion protein, is a protein or glycoprotein that is capable of being expressed by a host cell such that it localizes to the cell surface. Constructs, such as binding domain-immunoglobulin fusion proteins, that localize to the cell surface may do
10 so by virtue of having naturally present or artificially introduced structural features that direct the fusion protein to the cell surface (e.g., Nelson *et al.* 2001 *Trends Cell Biol.* 11:483; Ammon *et al.*, 2002 *Arch. Physiol. Biochem.* 110:137; Kasai *et al.*, 2001 *J. Cell Sci.* 114:3115; Watson *et al.*, 2001 *Am. J. Physiol. Cell Physiol.* 281:C215; Chatterjee *et al.*, 200 *J. Biol. Chem.* 275:24013) including by way of illustration and not limitation, secretory
15 signal sequences, leader sequences, plasma membrane anchor domain polypeptides and transmembrane domains such as hydrophobic transmembrane domains (e.g., Heuck *et al.*, 2002 *Cell Biochem. Biophys.* 36:89; Sadlish *et al.*, 2002 *Biochem J.* 364:777; Phoenix *et al.*, 2002 *Mol. Membr. Biol.* 19:1; Minke *et al.*, 2002 *Physiol. Rev.* 82:429) or glycosylphosphatidylinositol attachment sites (“glypiation” sites, e.g., Chatterjee *et al.*, 2001
20 *Cell Mol. Life Sci.* 58:1969; Hooper, 2001 *Proteomics* 1:748; Spiro, 2002 *Glycobiol.* 12:43R), cell surface receptor binding domains, extracellular matrix binding domains, or any other structural feature that causes at least a desired portion of the fusion protein population to localize, in whole or in part, to the cell surface. Particularly preferred are fusion protein constructs that comprise a plasma membrane anchor domain which includes a
25 transmembrane polypeptide domain, typically comprising a membrane spanning domain which includes a hydrophobic region capable of energetically favorable interaction with the phospholipid fatty acyl tails that form the interior of the plasma membrane bilayer. Such features are known to those of ordinary skill in the art, who will further be familiar with methods for introducing nucleic acid sequences encoding these features into the subject

expression constructs by genetic engineering, and with routine testing of such constructs to verify cell surface localization of the product.

According to certain further embodiments, a plasma membrane anchor domain polypeptide comprises such a transmembrane domain polypeptide and also comprises a cytoplasmic tail polypeptide, which refers to a region or portion of the polypeptide sequence that contacts the cytoplasmic face of the plasma membrane and/or is in contact with the cytosol or other cytoplasmic components. A large number of cytoplasmic tail polypeptides are known that comprise the intracellular portions of plasma membrane transmembrane proteins, and discrete functions have been identified for many such polypeptides, including biological signal transduction (*e.g.*, activation or inhibition of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides and other second messengers, ion channels, secretory pathways), biologically active mediator release, stable or dynamic association with one or more cytoskeletal components, cellular differentiation, cellular activation, mitogenesis, cytostasis, apoptosis and the like (*e.g.*, Maher *et al.*, 2002 *Immunol. Cell Biol.* **80**:131; El Far *et al.*, 2002 *Biochem J.* **365**:329; Teng *et al.*, 2002 *Genome Biol.* 2REVIEWS:3012; Simons *et al.*, 2001 *Cell Signal* **13**:855; Furie *et al.*, 2001 *Thromb. Haemost.* **86**:214; Gaffen, 2001 *Cytokine* **14**:63; Dittel, 2000 *Arch. Immunol. Ther. Exp.* (Warsz.) **48**:381; Parnes *et al.*, 2000 *Immunol. Rev.* **176**:75; Moretta *et al.*, 2000 *Semin. Immunol.* **12**:129; Ben Ze'ev, 1999 *Ann. N.Y. Acad. Sci.* **886**:37; Marsters *et al.*, *Recent Prog. Horm. Res.* **54**:225).

Figure 70 illustrates the binding, for example, of fluorocine-conjugated FcRIII (CD16) soluble fusion proteins to 2H7 scFv-binding domain constructs that are attached to CD20 expressed by cells, CHO cells in this example. CD16 binding to a construct of the invention, for example, scFv-binding domain construct, provides one example of a screening tool that may be used to detect and/or quantitate changes in CD16 binding to altered constructs of the invention, including scFv-binding domain constructs, that contains targeted or site-specific mutations, substitutions, deletions, or other alterations. Changes in CD16 binding properties may be reflected, for example, by changes in binding of either CD16 high affinity protein (158V) or CD16 low affinity protein (158 F) or both.

A schematic representation of one example of such a screening process is diagrammed in the second drawing in Figure 70, in which scFv-binding domain constructs are displayed on the cell surface of mammalian cells. scFv-binding domain molecules in this example are displayed on the cell surface through a molecule that serves as a transmembrane domain anchor. These molecules may represent, for example, a single scFv-binding domain construct or may be introduced into a population of mammalian cells as a library of such molecules. Transfected cells with altered binding properties can then, for example, be panned, sorted, or otherwise isolated from other cells by altering the stringency of the selection conditions and using CD16 fusion proteins as a binding probe. Cells that express scFv-Ig molecules with altered binding to either CD16 high affinity allele (158V) or CD16 low affinity allele (158F) or both, for example, can be isolated.

This display system can be used, for example, to create a library of constructs of the invention with mutated or otherwise altered tail regions with short stretches of CH2 sequence replaced with randomized oligonucleotides or, for example, randomization of a single residue with all possible amino acid substitutions, natural or unnatural, including synthetic amino acids. Once such a library is constructed, it can be transfected into appropriate cells, for example, COS cells, by methods known in the art. Transfectants can then be bound to, for example, labeled CD16 constructs, and panned or sorted based on their relative or desired binding properties to multiple allelotypes/isoforms. Desired cells may be harvested, and the DNA, for example, plasmid DNA, isolated and then transformed into, for example, bacteria. This process may be repeated iteratively multiple times until desired single clones are isolated from the mammalian host cells. See Seed B and Aruffo A, *Pro. Nat'l Acad Sci USA* 1987 **84**:3365-3369; Aruffo A and Seed B, *Pro. Nat'l Acad Sci USA* 1987 **84**:8573-8577.

One such use of this type of screening system, for example, is for the identification and/or isolation of constructs of the invention having tail regions, or tail regions, that bind equally well to both the high and low affinity alleles of CD16 with the goal of improving effector functions mediated by scFv-binding domain constructs in multiple subpopulations of patients. Constructs of the invention having tail regions, or tail regions with altered binding properties to other Fc receptors can also be selected using such

a display system, for example, the display system described. Other display systems that do not glycosylate proteins, for example, those that use bacteriophage or yeast, are not generally desired for selection of constructs of the invention having Ig-based tail regions, or Ig-based tail regions, with altered FcR binding properties. Most non-mammalian systems, for example, do not glycosylate proteins.

Expression of constructs of the invention, for example, scFv-binding domain constructs, expressed on the surface of a mammalian cell by incorporation of an appropriate molecule into the construct, for example, by incorporation of a transmembrane domain or a GPI anchor signal, also have utility in other display systems that are useful, for example, for selection of constructs of the invention, for example, altered scFv-binding domain molecules that will be produced at higher or other desired levels. In one such an embodiment, cells that are useful in the production of glycosylated proteins, for example, mammalian cells such as COS cells, are transfected with a library of scFv-binding domain constructs in a plasmid that directs their expression to the cell surface. Cells, such as COS cells, that express the highest or other desired level of the scFv-binding domain molecules are selected by techniques known in the art (for example panning, sterile cell sorting, magnetic bead separation, *etc.*), and DNA, for example, plasmid DNA, is isolated for transformation into other cells, for example, bacteria. After one or more rounds of selection single clones are isolated that encode scFv-binding domain molecules capable of a high or other desired level of expression. The isolated clones may then be altered to remove the membrane anchor and expressed in an appropriate cells system, for example, a mammalian cell system, wherein the scFv-binding domain constructs will be produced, for example, by secretion into the culture fluid at desired levels. Without being bound by any particular mechanism or theory, this is believed to result from the common requirement of secreted glycoproteins and cell surface glycoproteins for a signal peptide and processing through the golgi for expression. Thus, selection for a molecule that shows an improvement expression levels on a cell surface will also result in the identification of a molecule having an improvement in levels of secreted protein.

These display systems utilizing a construct of the invention may also be used for screening and/or identifying and/or isolating affinity variants of the binding domain within a construct.

Particularly preferred are such display and/or screening systems, for example, that include or use constructs that include (1) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, in a V_H region sequence (including in a V_H region sequence within an scFv or other construct), and/or (2) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence (including in a V_L region sequence within an scFv or other construct). Especially preferred are such display and/or screening systems that include or use constructs that include an engineered V_H sequence (whether or not associated with one or more other sequences, including immunoglobulin-derived and other sequences contained, for example, within an sFv or scFv-containing construct), which includes a mutation, alteration or deletion at an amino acid at a location or locations corresponding to amino acid position 11. The V_H11 amino acid, if substituted, may be substituted with another amino acid as described herein, or by another molecule as desired.

In the context of other methods of using constructs of the invention, including binding domain-immunoglobulin fusion proteins, for the treatment of a malignant condition or a B cell disorder(s) as provided herein, including, for example, by one or more of a number of gene therapy methods and related construct delivery techniques, the present invention also contemplates certain embodiments wherein a construct, for example, a binding domain-immunoglobulin fusion protein that comprises a plasma membrane anchor domain polypeptide is expressed (or capable of expression) at a cell surface and may further comprise a cytoplasmic tail polypeptide which comprises an apoptosis signaling polypeptide

sequence. A number of apoptosis signaling polypeptide sequences are known to the art, as reviewed, for example, in *When Cells Die: A Comprehensive Evaluation of Apoptosis and Programmed Cell Death* (R.A. Lockshin *et al.*, Eds., 1998 John Wiley & Sons, New York; see also, *e.g.*, Green *et al.*, 1998 *Science* **281**:1309 and references cited therein; Ferreira *et al.*, 2002 *Clin. Canc. Res.* **8**:2024; Gurumurthy *et al.*, 2001 *Cancer Metastas. Rev.* **20**:225; Kanduc *et al.*, 2002 *Int. J. Oncol.* **21**:165). Typically an apoptosis signaling polypeptide sequence comprises all or a portion of, or is derived or constructed from, a receptor death domain polypeptide, for instance, FADD (*e.g.*, Genbank Acc. Nos. U24231, U43184, AF009616, AF009617, NM_012115), TRADD (*e.g.*, Genbank Acc. No. NM_003789), RAIDD (*e.g.*, Genbank Acc. No. U87229), CD95 (FAS/Apo-1; *e.g.*, Genbank Acc. Nos. X89101, NM_003824, AF344850, AF344856), TNF- α -receptor-1 (TNFR1, *e.g.*, Genbank Acc. Nos. S63368, AF040257), DR5 (*e.g.*, Genbank Acc. No. AF020501, AF016268, AF012535), an ITIM domain (*e.g.*, Genbank Acc. Nos. AF081675, BC015731, NM_006840, NM_006844, NM_006847, XM_017977; see, *e.g.*, Billadeau *et al.*, 2002 *J. Clin. Invest.* **109**:161), an ITAM domain (*e.g.*, Genbank Acc. Nos. NM_005843, NM_003473, BC030586; see, *e.g.*, Billadeau *et al.*, 2002), or other apoptosis-associated receptor death domain polypeptides known to the art, for example, TNFR2 (*e.g.*, Genbank Acc. No. L49431, L49432), caspase/procaspase-3 (*e.g.*, Genbank Acc. No. XM_54686), caspase/procaspase-8 (*e.g.*, AF380342, NM_004208, NM_001228, NM_033355, NM_033356, NM_033357, NM_033358), caspase/procaspase-2 (*e.g.*, Genbank Acc. No. AF314174, AF314175), *etc.*

Cells in a biological sample that are suspected of undergoing apoptosis may be examined for morphological, permeability or other changes that are indicative of an apoptotic state. For example by way of illustration and not limitation, apoptosis in many cell types may cause altered morphological appearance such as plasma membrane blebbing, cell shape change, loss of substrate adhesion properties or other morphological changes that can be readily detected by a person having ordinary skill in the art, for example by using light microscopy. As another example, cells undergoing apoptosis may exhibit fragmentation and disintegration of chromosomes, which may be apparent by microscopy and/or through the use of DNA-specific or chromatin-specific dyes that are known in the art,

including fluorescent dyes. Such cells may also exhibit altered plasma membrane permeability properties as may be readily detected through the use of vital dyes (*e.g.*, propidium iodide, trypan blue) or by the detection of lactate dehydrogenase leakage into the extracellular milieu. These and other means for detecting apoptotic cells by morphologic
5 criteria, altered plasma membrane permeability, and related changes will be apparent to those familiar with the art.

In another embodiment of the invention wherein a construct, such as a binding domain-immunoglobulin fusion protein, that is expressed at a cell surface comprises a plasma membrane anchor domain having a transmembrane domain and a cytoplasmic tail
10 that comprises an apoptosis signaling polypeptide, cells in a biological sample may be assayed for translocation of cell membrane phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane, which may be detected, for example, by measuring outer leaflet binding by the PS-specific protein annexin. Martin *et al.*, *J. Exp. Med.* **182**:1545, 1995; Fadok *et al.*, *J. Immunol.* **148**:2207, 1992. In still other related
15 embodiments of the invention, including embodiments wherein a construct, such as a binding domain-immunoglobulin fusion protein, is expressed at the cell surface and comprises a plasma membrane anchor domain having an apoptosis signaling polypeptide and also including embodiments wherein the construct, such as a binding domain-immunoglobulin fusion protein, is a soluble protein that lacks a membrane anchor domain
20 and that is capable of inducing apoptosis, a cellular response to an apoptogen is determined by an assay for induction of specific protease activity in any member of a family of apoptosis-activated proteases known as the caspases (see, *e.g.*, Green *et al.*, 1998 *Science* **281**:1309). Those having ordinary skill in the art will be readily familiar with methods for determining caspase activity, for example by determination of caspase-mediated cleavage of
25 specifically recognized protein substrates. These substrates may include, for example, poly-(ADP-ribose) polymerase (PARP) or other naturally occurring or synthetic peptides and proteins cleaved by caspases that are known in the art (*see, e.g.*, Ellerby *et al.*, 1997 *J. Neurosci.* **17**:6165). The synthetic peptide Z-Tyr-Val-Ala-Asp-AFC (SEQ ID NO:__), wherein "Z" indicates a benzoyl carbonyl moiety and AFC indicates 7-amino-4-trifluoromethylcoumarin (Kluck *et al.*, 1997 *Science* **275**:1132; Nicholson *et al.*, 1995
30

Nature 376:37), is one such substrate. Other non-limiting examples of substrates include nuclear proteins such as U1-70 kDa and DNA-PKcs (Rosen and Casciola-Rosen, 1997 *J. Cell. Biochem.* 64:50; Cohen, 1997 *Biochem. J.* 326:1). Cellular apoptosis may also be detected by determination of cytochrome c that has escaped from mitochondria in apoptotic cells (*e.g.*, Liu *et al.*, *Cell* 86:147, 1996). Such detection of cytochrome c may be performed spectrophotometrically, immunochemically or by other well established methods for determining the presence of a specific protein. Persons having ordinary skill in the art will readily appreciate that there may be other suitable techniques for quantifying apoptosis.

Particularly preferred embodiments of constructs useful for gene therapy applications, including those constructs that include a plasma membrane anchor domain and/or cytoplasmic tail polypeptide (including, for example, an apoptosis signaling sequence), are such constructs that include (1) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, in a V_H region sequence (including in a V_H region sequence within an scFv or other construct), and/or (2) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence (including in a V_L region sequence within an scFv or other construct). Especially preferred are constructs that include an engineered V_H sequence (whether or not associated with one or more other sequences, including immunoglobulin-derived and other sequences contained, for example, within an sFv or scFv-containing construct), which includes a mutation, alteration or deletion at an amino acid at a location or locations corresponding to amino acid position 11. The V_H11 amino acid, if substituted, may be substituted with another amino acid as described herein, or by another molecule as desired.

Once a construct, such as for example a binding domain-immunoglobulin fusion protein, as provided herein has been designed, polynucleotides including DNAs

encoding the construct, where it or a relevant portion of it is a polypeptide, may be synthesized in whole or in part via oligonucleotide synthesis as described, for example, in Sinha *et al.*, *Nucleic Acids Res.*, **12**:4539-4557 (1984); assembled via PCR as described, for example in Innis, Ed., *PCR Protocols*, Academic Press (1990) and also in Better *et al.* *J. Biol. Chem.* **267**:16712-16118 (1992); cloned and expressed via standard procedures as described, for example, in Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1989) and also in Robinson *et al.*, *Hum. Antibod. Hybridomas*, **2**:84-93 (1991); and tested for desired activity, for example, binding to a target, or specific antigen binding activity, as described, for example, in Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988) and Munson *et al.*, *Anal. Biochem.*, **107**:220-239 (1980).

The preparation of single polypeptide chain binding molecules of the Fv region, single-chain Fv molecules, is known in the art. See, *e.g.*, U.S. Pat. No. 4,946,778. In the present invention, single-chain Fv-like molecules that may be included in constructs of the invention may be synthesized by encoding a first variable region of the heavy or light chain, followed by one or more linkers to the variable region of the corresponding light or heavy chain, respectively. The selection of various appropriate linker(s) between the two variable regions is described in U.S. Pat. No. 4,946,778 (see also, *e.g.*, Huston *et al.*, 1993 *Int. Rev. Immunol.* **10**:195). An exemplary linker described herein is (Gly-Gly-Gly-Gly-Ser)₃, but may be of any desired length. The linker is used to convert the naturally aggregated but chemically separate heavy and light chains into the amino terminal antigen binding portion of a single polypeptide chain, for example, wherein this antigen binding portion will fold into a structure similar to the original structure made of two polypeptide chains, or that otherwise has the ability to bind to a target, for example a target antigen. For those constructs that include an scFv as a binding region, a native or engineered immunoglobulin hinge as a connecting region, and one or more native or engineered heavy chain constant regions as a binding region, nucleotide sequences encoding the variable regions of native or engineered heavy and light chains, joined by a sequence encoding a linker, are joined to a nucleotide sequence encoding native or engineered antibody constant regions, as desired. The constant regions may be those that permit the resulting polypeptide

to form interchain disulfide bonds to form a dimer, and which contain desired effector functions, such as the ability to mediate ADCC, CDC, or fix complement, although native or engineered constant regions that do not favor dimer or other multimer formation or aggregation are preferred.. For a construct, such as an immunoglobulin-like molecule, of the invention that is intended for use in humans, the included sequences having constant regions and/or desired constant regions function(s) will typically be human or substantially human or humanized to minimize a potential anti-human immune response and to provide appropriate or desired effector functions. Manipulation of sequences encoding antibody constant regions is referenced in the PCT publication of Morrison and Oi, WO 89/07142. In preferred embodiments, the CH1 domain is deleted in whole or in part from a tail region that includes, or consists essentially of, or consists of, a native or engineered immunoglobulin constant region(s) (for example, native or engineered CH2 and/or CH3 constant region(s), or native or engineered CH2 and/or CH3 and/or CH4 constant region(s)), and the carboxyl end of the binding region, for example, a binding domain polypeptide such as an immunoglobulin variable region polypeptide, is joined to the amino terminus of, for example, a CH2 via a connecting region, for example, a native or engineered hinge region polypeptide as provided herein.

As described above, the present invention provides recombinant expression constructs capable of directing the expression of constructs of the invention, including binding domain-immunoglobulin fusion proteins, as provided herein. The amino acids, which occur in the various amino acid sequences referred to herein, are identified according to their well known three-letter or single-letter abbreviations. The nucleotides, which occur in the various DNA sequences or fragments thereof referred herein, are designated with the standard single letter designations used routinely in the art. A given amino acid sequence may also encompass similar but changed amino acid sequences, such as those having only minor changes, for example by way of illustration and not limitation, covalent chemical modifications, insertions, deletions and substitutions, which may further include conservative substitutions or substitutions with non-naturally-occurring amino acids. Amino acid sequences that are similar to one another may share substantial regions of sequence homology. In like fashion, nucleotide sequences may encompass substantially similar

nucleotide sequences having only minor changes, for example by way of illustration and not limitation, covalent chemical modifications, insertions, deletions and substitutions, which may further include silent mutations owing to degeneracy of the genetic code. Nucleotide sequences that are similar to one another may share substantial regions of sequence
5 homology.

The presence of a malignant condition in a subject refers to the presence of dysplastic, cancerous, and/or transformed cells in the subject, including, for example, neoplastic, tumor, non-contact inhibited, or oncogenically transformed cells, or the like (*e.g.*, melanoma, carcinomas such as adenocarcinoma, squamous cell carcinoma, small cell
10 carcinoma, oat cell carcinoma, *etc.*, sarcomas such as chondrosarcoma, osteosarcoma, *etc.*) which are known to the art and for which criteria for diagnosis and classification are established. In preferred embodiments contemplated by the present invention, for example, such cancer cells are malignant hematopoietic cells, such as transformed cells of lymphoid lineage and in particular, B cell lymphomas and the like; cancer cells may in certain
15 preferred embodiments also be epithelial cells such as carcinoma cells. The invention also contemplates B cell disorders, which may include certain malignant conditions that affect B cells (*e.g.*, B cell lymphoma) but which is not intended to be so limited, and which is also intended to encompass autoimmune diseases and in particular, diseases, disorders and conditions that are characterized by autoantibody production, for example.

20 Autoantibodies are antibodies that react with self antigens. Autoantibodies are detected in several autoimmune diseases (*i.e.*, a disease, disorder or condition wherein a host immune system generates an inappropriate anti-“self” immune reaction) where they are involved in disease activity. Current treatments for various autoimmune diseases include immunosuppressive drugs that require continuing administration, lack specificity, and cause
25 significant side effects. New approaches that can eliminate autoantibody production with minimal toxicity will address an unmet medical need for a spectrum of diseases that affect many people. Constructs of the subject invention, including binding domain-immunoglobulin fusion proteins, are designed, for example, for improved penetration into lymphoid tissues. Depletion of B lymphocytes interrupts the autoantibody production cycle,

and allows the immune system to reset as new B lymphocytes are produced from precursors in the bone marrow.

A number of diseases, disorders, and conditions have been identified for which beneficial effects are believed, according to non-limiting theory, to result from B cell depletion therapy. Such diseases disorders, and conditions include, but are not limited to, Grave's disease, Hashimoto's disease, rheumatoid arthritis, systemic lupus erythematosus, Sjogrens Syndrome Immune Thrombocytopenic purpura, multiple sclerosis, myasthenia gravis, scleroderma, psoriasis, Inflammatory Bowel Disease including Crohn's disease and ulcerative colitis, .. Inflammatory Bowel Disease including Crohn's disease and Ulcerative colitis, are autoimmune diseases of the digestive system.

The present invention further relates to nucleotide constructs encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, and in particular to methods for administering recombinant constructs encoding such proteins for gene therapy applications that may be expressed, for example, as fragments, analogs and derivatives of such polypeptides.

The terms "fragment," "derivative" and "analog" when referring to constructs of the invention including, for example, binding domain-immunoglobulin fusion polypeptides or fusion proteins, refers to any construct, such as a binding domain-immunoglobulin fusion polypeptide or fusion protein, that retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a pro- or prepro- form of a construct, for example, a pro-protein that can be activated by cleavage of the pro-protein portion to produce an active construct, such as a binding domain-immunoglobulin fusion polypeptide.

A fragment, derivative or analog of a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide or fusion protein, including binding domain-immunoglobulin fusion polypeptides or fusion proteins encoded by the cDNAs referred to herein, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a

substituent group, or (iii) one in which additional amino acids are fused or otherwise connected to the construct, *e.g.*, a binding domain-immunoglobulin fusion polypeptide, including amino acids that are employed for detection or specific functional alteration of the construct, including such constructs as a binding domain-immunoglobulin fusion polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The constructs, including polypeptide constructs, of the present invention include, for example, binding domain-immunoglobulin fusion polypeptides and fusion proteins having binding regions such as binding domain polypeptide amino acid sequences that are identical or similar to sequences known in the art, or fragments or portions thereof. For example by way of additional illustration and not limitation, a human CD154 molecule extracellular domain [SEQ ID NO:___] is contemplated for use according to the instant invention, as are portions of such polypeptides and/or polypeptides having at least about 70% similarity (preferably greater than a 70% identity) and more preferably about 90% similarity (more preferably greater than a 90% identity) to the reported polypeptide and still more preferably about 95% similarity (still more preferably greater than a 95% identity) to the reported polypeptides and to portions of such polypeptides, wherein such portions of a binding domain-immunoglobulin fusion polypeptide, for example, generally contain at least about 30 amino acids and more preferably at least about 50 amino acids. Extracellular domains include, for example, portions of a cell surface molecule, and in particularly preferred embodiments cell surface molecules that are integral membrane proteins or that comprise a plasma membrane spanning transmembrane domain, that are constructed to extend beyond the outer leaflet of the plasma membrane phospholipid bilayer when the molecule is expressed at a cell surface, preferably in a manner that exposes the extracellular domain portion of such a molecule to the external environment of the cell, also known as the extracellular milieu. Methods for determining whether a portion of a cell surface molecule comprises an extracellular domain are well known to the art and include, for example, experimental determination (*e.g.*, direct or indirect labeling of the molecule, evaluation of whether the molecule can be structurally altered by agents to which the plasma membrane is not permeable such as proteolytic or lipolytic enzymes) or topological prediction based on

the structure of the molecule (*e.g.*, analysis of the amino acid sequence of a polypeptide) or other methodologies.

As used herein, an “amino acid” is a molecule having the structure wherein a central carbon atom (the alpha (α)-carbon atom) is linked to a hydrogen atom, a carboxylic acid group (the carbon atom of which is referred to herein as a “carboxyl carbon atom”), an amino group (the nitrogen atom of which is referred to herein as an “amino nitrogen atom”), and a side chain group, R. When incorporated into a peptide, polypeptide, or protein, an amino acid loses one or more atoms of its amino and carboxylic groups in the dehydration reaction that links one amino acid to another. As a result, when incorporated into a protein, an amino acid may also be referred to as an “amino acid residue.” In the case of naturally occurring proteins, an amino acid residue’s R group differentiates the 20 amino acids from which proteins are typically synthesized, although one or more amino acid residues in a protein may be derivatized or modified following incorporation into protein in biological systems (*e.g.*, by glycosylation and/or by the formation of cystine through the oxidation of the thiol side chains of two non-adjacent cysteine amino acid residues, resulting in a disulfide covalent bond that frequently plays an important role in stabilizing the folded conformation of a protein, *etc.*). As those in the art will appreciate, non-naturally occurring amino acids can also be incorporated into proteins, particularly those produced by synthetic methods, including solid state and other automated synthesis methods: Examples of such amino acids include, without limitation, α -amino isobutyric acid, 4-amino butyric acid, L-amino butyric acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteine, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids (*e.g.*, β -methyl amino acids, α -methyl amino acids, N α -methyl amino acids) and amino acid analogs in general. In addition, when an α -carbon atom has four different groups (as is the case with the 20 amino acids used by biological systems to synthesize proteins, except for glycine, which has two hydrogen atoms bonded to the α carbon atom), two different enantiomeric forms of each amino acid exist, designated D and L. In mammals, only L-amino acids are incorporated into naturally occurring polypeptides.

The instant invention envisions proteins incorporating one or more D- and L- amino acids, as well as proteins comprised of just D- or L- amino acid residues.

Herein, the following abbreviations may be used for the following amino acids (and residues thereof): alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cyteine (Cys, C); glycine (Gly, G); glutamic acid (Glu, E); glutamine (Gln, Q); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V). Non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionines. Neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, esparagine, and glutamine. Positively charged (basic amino acids include arginine, lysine and histidine. Negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

“Protein” refers to any polymer of two or more individual amino acids (whether or not naturally occurring) linked via a peptide bond, and occurs when the carboxyl carbon atom of the carboxylic acid group bonded to the α -carbon of one amino acid (or amino acid residue) becomes covalently bound to the amino nitrogen atom of amino group bonded to the α -carbon of an adjacent amino acid. The term “protein” is understood to include the terms “polypeptide” and “peptide” (which, at times, may be used interchangeably herein) within its meaning. In addition, proteins comprising multiple polypeptide subunits or other components will also be understood to be included within the meaning of “protein” as used herein. Similarly, fragments of proteins, peptides, and polypeptides are also within the scope of the invention and may be referred to herein as “proteins.”

In biological systems (be they *in vivo* or *in vitro*, including cell-free, systems), the particular amino acid sequence of a given protein (*i.e.*, the polypeptide’s “primary structure,” when written from the amino-terminus to carboxy-terminus) is determined by the nucleotide sequence of the coding portion of a mRNA, which is in turn specified by genetic information, typically genomic DNA (which, for purposes of this invention, is understood to include organelle DNA, for example, mitochondrial DNA and

chloroplast DNA). Of course, any type of nucleic acid which constitutes the genome of a particular organism (*e.g.*, double-stranded DNA in the case of most animals and plants, single or double-stranded RNA in the case of some viruses, *etc.*) is understood to code for the gene product(s) of the particular organism. Messenger RNA is translated on a ribosome, which catalyzes the polymerization of a free amino acid, the particular identity of which is specified by the particular codon (with respect to mRNA, three adjacent A, G, C, or U ribonucleotides in the mRNA's coding region) of the mRNA then being translated, to a nascent polypeptide. Recombinant DNA techniques have enabled the large-scale synthesis of proteins and polypeptides (*e.g.*, human insulin, human growth hormone, erythropoietin, granulocyte colony stimulating factor, *etc.*) having the same primary sequence as when produced naturally in living organisms. In addition, such technology has allowed the synthesis of analogs of these and other proteins, which analogs may contain one or more amino acid deletions, insertions, and/or substitutions as compared to the native proteins. Recombinant DNA technology also enables the synthesis of entirely novel proteins.

In non-biological systems (*e.g.*, those employing solid state synthesis), the primary structure of a protein (which also includes disulfide (cystine) bond locations) can be determined by the user. As a result, polypeptides having a primary structure that duplicates that of a biologically produced protein can be achieved, as can analogs of such proteins. In addition, completely novel polypeptides can also be synthesized, as can protein incorporating non-naturally occurring amino acids.

As is known in the art, "similarity" between two polypeptides may be determined by comparing the amino acid sequence (including conserved amino acid substitutes therein) of one polypeptide to the sequence of a second polypeptide. Fragments or portions of the nucleic acids encoding polypeptides of the present invention may be used to synthesize full-length nucleic acids of the present invention. As used herein, "% identity" refers to the percentage of identical amino acids situated at corresponding amino acid residue positions when two or more polypeptide are aligned and their sequences analyzed using, for example, a gapped BLAST algorithm (*e.g.*, Altschul *et al.*, 1997 *Nucl. Ac. Res.* **25**:3389; Altschul *et al.*, 1990 *J. Mol. Biol.*, **215**:403-410) which weights sequence gaps and sequence mismatches according to the default weightings provided by the National Institutes

of Health/ NCBI database (Bethesda, MD; *see* www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast). Other alignment methods include BLITZ (MPsrch) (Sturrock & Collins, 1993), and FASTA (Pearson and Lipman, 1988 *Proc. Natl. Acad. Sci. USA.* **85**:2444-2448).

The term “isolated” means, in the case of a naturally occurring material, that the material is or has been removed from, or is no longer associated with, its natural or original environment. For example, a naturally occurring nucleic acid or protein or polypeptide present in a living animal is not isolated, but the same nucleic acid or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated. Such nucleic acids could be part of a vector and/or such nucleic acids or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The term “isolated”, in the case of non-naturally occurring material, such as a recombinantly manufactured construct of the invention, includes material that is substantially or essentially free from components which normally accompany it during manufacture, such as, for example, proteins and peptides that have been purified to a desired degree, preferably, for example, so that they are at least about 80% pure, more preferably at least about 90%, and still more preferably at least about 95% as measured by techniques known in the art.

The term “gene” means a segment of DNA involved in producing a polypeptide chain; it may also include regions preceding and following a polypeptide coding region, for example, a “leader and trailer” as well as intervening sequences (introns) between relevant individual coding segments (exons).

As described herein, the invention provides constructs, including binding domain-immunoglobulin fusion proteins, that may be encoded in whole or in part by nucleic acids that have a binding region coding sequence such as, for example, a binding domain coding sequence fused or otherwise connected in frame to an additional native or engineered immunoglobulin domain encoding sequence to provide for expression of, for example, a binding domain polypeptide sequence fused or otherwise connected to an additional functional polypeptide sequence that permits, for example by way of illustration and not limitation, detection, functional alteration, isolation and/or purification of the fusion protein. Such fusion proteins may permit functional alteration of a binding domain by containing

additional immunoglobulin-derived polypeptide sequences that influence behavior of the fusion product, for example (and as described above) by reducing the availability of sulfhydryl groups for participation in disulfide bond formation, and by conferring the ability to potentiate ADCC and/or CDC and/or fix complement.

5 Modification of a polypeptide may be effected by any means known to those of skill in this art. The preferred methods herein rely on modification of DNA encoding, for example, a fusion protein and expression of the modified DNA. DNA encoding one of the constructs of the invention, for example, one of the binding domain-immunoglobulin fusions discussed herein, for example, may be altered or mutagenized using standard methodologies,
10 including those described below. For example, cysteine residues that may otherwise facilitate multimer formation or promote particular molecular conformations can be deleted from a polypeptide or replaced, *e.g.*, cysteine residues that are responsible for or participate in aggregate formation. If necessary, for example, the identity of cysteine residues that contribute to aggregate formation may be determined empirically, by deleting and/or
15 replacing a cysteine residue and ascertaining whether the resulting protein aggregates in solutions containing physiologically acceptable buffers and salts. In addition, fragments of, for example, binding domain-immunoglobulin fusions may be constructed and used. As noted above, counterreceptor/ligand binding domains for many candidate binding domain-immunoglobulin fusion have been delineated, such that one having ordinary skill in the art may readily select appropriate polypeptide domains for inclusion in encoded products of the
20 instant expression constructs.

Conservative substitutions of amino acids are well-known and may be made generally without altering the biological activity of the resulting binding domain-immunoglobulin fusion protein molecule. For example, such substitutions are generally
25 made by interchanging within the groups of polar residues, charged residues, hydrophobic residues, small residues, and the like. If necessary, such substitutions may be determined empirically merely by testing the resulting modified protein for the ability to bind to the appropriate cell surface receptors in *in vitro* biological assays, or to bind to appropriate antigens or desired target molecules.

The present invention further relates to nucleic acids which hybridize to constructs of the invention, including for example, binding domain-immunoglobulin fusion protein encoding polynucleotide sequences as provided herein, or their complements, as will be readily apparent to those familiar with the art, if there is at least about 70%, preferably at least about 80-85%, more preferably at least about 90%, and still more preferably at least about 95%, 96%, 97%, 98% or 99% identity between the sequences.

The present invention particularly relates to nucleic acids which hybridize under stringent conditions to, for example, the binding domain-immunoglobulin fusion encoding nucleic acids referred to herein. As used herein, to "hybridize" under conditions of a specified stringency is used to describe the stability of hybrids formed between two single-stranded nucleic acid molecules. Stringency of hybridization is typically expressed in conditions of ionic strength and temperature at which such hybrids are annealed and washed. The term "stringent conditions" refers to conditions that permit hybridization between polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent (*e.g.*, formamide), temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of organic solvents (*e.g.*, formamide), or raising the hybridization temperature. For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, *e.g.*, formamide, while high stringency hybridization can be obtained in the presence of an organic solvent (*e.g.*, at least about 35% formamide, most preferably at least about 50% formamide). Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, for example, hybridization time, the concentration of detergent, *e.g.*, sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed, and are within the skill in the art. Other typical "high", "medium" and "low" stringency encompass

the following conditions or equivalent conditions thereto: high stringency: 0.1 x SSPE or SSC, 0.1% SDS, 65°C; medium stringency: 0.2 x SSPE or SSC, 0.1% SDS, 50°C; and low stringency: 1.0 x SSPE or SSC, 0.1% SDS, 50°C. As known to those having ordinary skill in the art, variations in stringency of hybridization conditions may be achieved by altering the time, temperature and/or concentration of the solutions used for prehybridization, hybridization and wash steps, and suitable conditions may also depend in part on the particular nucleotide sequences of the probe used, and of the blotted, proband nucleic acid sample. Accordingly, it will be appreciated that suitably stringent conditions can be readily selected without undue experimentation where a desired selectivity of the probe is identified, based on its ability to hybridize to one or more certain proband sequences while not hybridizing to certain other proband sequences.

As used herein, preferred "stringent conditions" generally refer to hybridization that will occur only if there is at least about 90-95% and more preferably at least about 97% identity between the sequences. The nucleic acid constructs which hybridize to, for example, binding domain-immunoglobulin fusion encoding nucleic acids referred to herein, in preferred embodiments, encode polypeptides which retain substantially the same biological function or activity as, for example, the binding domain-immunoglobulin fusion polypeptides encoded by the cDNAs.

The nucleic acids of the present invention, also referred to herein as polynucleotides, may be in the form of RNA, for example, mRNA, or in the form of DNA, which DNA includes cDNA (also called "complementary DNA", which is a DNA molecule that is complementary to a specific messenger RNA), genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. A coding sequence which encodes a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide for use according to the invention may contain portions that are identical to the coding sequence known in the art or described herein for portions thereof, or may be a different coding sequence, which, as a result of the redundancy or degeneracy of the genetic code, encodes the same construct or portion thereof, including all or a portion of a binding domain-immunoglobulin fusion polypeptide.

The nucleic acids which encode constructs of the invention, for example, binding domain-immunoglobulin fusion polypeptides, for use according to the invention may include, but are not limited to: only the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide; the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide and additional coding sequence; the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequences 5' and/or 3' of the coding sequence for the binding domain-immunoglobulin fusion polypeptide or a portion(s) thereof, which for example may further include but need not be limited to one or more regulatory nucleic acid sequences that may be a regulated or regulatable promoter, enhancer, other transcription regulatory sequence, repressor binding sequence, translation regulatory sequence or any other regulatory nucleic acid sequence. Thus, the term "nucleic acid encoding" or "polynucleotide encoding" a construct, for example, a binding domain-immunoglobulin fusion protein, encompasses a nucleic acid which includes only coding sequence for, for example, a binding domain-immunoglobulin fusion polypeptide as well as a nucleic acid which includes additional coding and/or non-coding sequence(s).

Nucleic acids and oligonucleotides for use as described herein can be synthesized by any method known to those of skill in this art (*see, e.g.*, WO 93/01286, U.S. Application Serial No. 07/723,454; U.S. Patent No. 5,218,088; U.S. Patent No. 5,175,269; U.S. Patent No. 5,109,124). Identification of various oligonucleotides and nucleic acid sequences also involves methods known in the art. For example, the desirable properties, lengths and other characteristics of oligonucleotides useful for cloning are well known. In certain embodiments, synthetic oligonucleotides and nucleic acid sequences may be designed that resist degradation by endogenous host cell nucleolytic enzymes by containing such linkages as: phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages that have proven useful in antisense applications. *See, e.g.*, Agrwal *et al.*, *Tetrahedron Lett.* **28**:3539-3542 (1987); Miller *et al.*, *J. Am. Chem. Soc.* **93**:6657-6665 (1971); Stec *et al.*, *Tetrahedron Lett.* **26**:2191-2194 (1985); Moody *et al.*, *Nucl. Acids Res.* **12**:4769-4782 (1989); Uznanski *et*

al., *Nucl. Acids Res.* (1989); Letsinger *et al.*, *Tetrahedron* **40**:137-143 (1984); Eckstein, *Annu. Rev. Biochem.* **54**:367-402 (1985); Eckstein, *Trends Biol. Sci.* **14**:97-100 (1989); Stein In: *Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression*, Cohen, Ed, Macmillan Press, London, pp. 97-117 (1989); Jager *et al.*, *Biochemistry* **27**:7237-7246 (1988).

5 In one embodiment, the present invention provides truncated components (*e.g.*, binding domain polypeptide, hinge region polypeptide, linker, *etc.*) for use in a construct of the invention, for example, a binding domain-immunoglobulin fusion protein. In another embodiment the invention provides nucleic acids encoding a construct of the invention, for example, a binding domain-immunoglobulin fusion protein having such
10 truncated components. A truncated molecule may be any molecule that comprises less than a full length version of the molecule of interest. Truncated molecules provided by the present invention may include truncated biological polymers, and in preferred embodiments of the invention such truncated molecules may be truncated nucleic acid molecules or truncated polypeptides. Truncated nucleic acid molecules have less than the full length
15 nucleotide sequence of a known or described nucleic acid molecule, where such a known or described nucleic acid molecule may be a naturally occurring, a synthetic, or a recombinant nucleic acid molecule, so long as one skilled in the art would regard it as a full length molecule. Thus, for example, truncated nucleic acid molecules that correspond to a gene sequence contain less than the full length gene where the gene comprises coding and non-
20 coding sequences, promoters, enhancers and other regulatory sequences, flanking sequences and the like, and other functional and non-functional sequences that are recognized as part of the gene. In another example, truncated nucleic acid molecules that correspond to a mRNA sequence contain less than the full length mRNA transcript, which may include various translated and non-translated regions as well as other functional and non-functional
25 sequences.

 In other preferred embodiments, truncated molecules are polypeptides that comprise less than the full length amino acid sequence of a particular protein or polypeptide component. As used herein "deletion" has its common meaning as understood by those familiar with the art, and may refer to molecules that lack one or more portions of a
30 sequence from either terminus or from a non-terminal region, relative to a corresponding full

length molecule, for example, as in the case of truncated molecules provided herein. Truncated molecules that are linear biological polymers such as nucleic acid molecules or polypeptides may have one or more of a deletion from either terminus of the molecule and/or one or more deletions from a non-terminal region of the molecule, where such deletions may be deletions of from about 1-1500 contiguous nucleotide or amino acid residues, preferably about 1-500 contiguous nucleotide or amino acid residues and more preferably about 1-300 contiguous nucleotide or amino acid residues, including deletions of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31-40, 41-50, 51-74, 75-100, 101-150, 151-200, 201-250 or 251-299 contiguous nucleotide or amino acid residues. In certain particularly preferred embodiments truncated nucleic acid molecules may have a deletion of about 270-330 contiguous nucleotides. In certain more preferred embodiments, truncated polypeptide molecules may have a deletion, for example, of about 80-140 contiguous amino acids.

The present invention further relates to variants of the herein referenced nucleic acids that encode fragments, analogs and/or derivatives of a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide. The variants of the nucleic acids encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, may be naturally occurring allelic variants of one or more portions of the the nucleic acid sequences included therein, or non-naturally occurring variants of such sequences or portions or sequences, including sequences varied by molecular engineering using, for example, methods known in the art for varying sequence. As is known in the art, an allelic variant is an alternate form of a nucleic acid sequence which may have at least one of a substitution, a deletion or an addition of one or more nucleotides, any of which does not substantially or undesireably alter the function of the encoded binding domain-immunoglobulin fusion polypeptide.

Variants and derivatives of constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, may be obtained by mutations of nucleotide sequences encoding, for example, binding domain-immunoglobulin fusion polypeptides or any portion thereof. Alterations of the native amino acid sequence may be accomplished by any of a number of conventional methods. Mutations can be introduced at particular loci,

for example, by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

5 Alternatively, for example, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene wherein predetermined codons can be altered by substitution, deletion or insertion. Exemplary methods of making such alterations are disclosed by Walder *et al.*, 1986 *Gene* 42:133; Bauer *et al.*, 1985 *Gene* 37:73; Craik, January 1985 *BioTechniques* 12-19; Smith *et al.*, January 1985 *Genetic Engineering: Principles and Methods BioTechniques* 12-19; Costa GL, *et al.*, "Site-directed mutagenesis using a rapid PCR-based method," 1996 *Methods Mol Biol.* 57:239-48; Rashtchian A., "Novel methods for cloning and engineering genes using the polymerase chain reaction," 1995 *Curr Opin Biotechnol.* 6(1):30-6; Sharon J, *et al.*, "Oligonucleotide-directed mutagenesis of antibody combining sites," 1993 *Int Rev Immunol.* 10(2-3):113-27; Kunkel, 10 1985 *Proc. Natl. Acad. Sci. USA* 82:488; Kunkel *et al.*, 1987 *Methods in Enzymol.* 154:367; 15 and, U.S. Patent Nos. 4,518,584 and 4,737,462.

As an example, modification of DNA may be performed by site-directed mutagenesis of DNA encoding a protein combined with the use of DNA amplification methods using primers to introduce and amplify alterations in the DNA template, such as 20 PCR splicing by overlap extension (SOE). Site-directed mutagenesis is typically effected using a phage vector that has single- and double-stranded forms, such as M13 phage vectors, which are well-known and commercially available. Other suitable vectors that contain a single-stranded phage origin of replication may be used. See, *e.g.*, Veira *et al.*, 1987 *Meth. Enzymol.* 15:3. In general, site-directed mutagenesis is performed by preparing a single- 25 stranded vector that encodes the protein of interest (*e.g.*, all or a component portion of a given binding domain-immunoglobulin fusion protein). An oligonucleotide primer that contains the desired mutation within a region of homology to the DNA in the single-stranded vector is annealed to the vector followed by addition of a DNA polymerase, such as *E. coli* DNA polymerase I (Klenow fragment), which uses the double stranded region as a 30 primer to produce a heteroduplex in which one strand encodes the altered sequence and the

other the original sequence. The heteroduplex is introduced into appropriate bacterial cells and clones that include the desired mutation are selected. The resulting altered DNA molecules may be expressed recombinantly in appropriate host cells to produce the modified protein.

5 Equivalent DNA constructs that include code for additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences not needed or desired for biological activity, for example, are also encompassed by the invention. For example, and as discussed above, sequences encoding Cys residues that are not desirable or essential for biological activity can be altered to cause the Cys residues to be
10 deleted or replaced with other amino acids, for example, thus preventing formation of incorrect or undesired intramolecular disulfide bridges upon synthesis or renaturation.

 A "host cell" or "recombinant host cell" is a cell that contains a vector, *e.g.*, an expression vector, or a cell that has otherwise been manipulated by recombinant techniques to express a protein of interest. Host organisms include those organisms in
15 which recombinant production of constructs of the invention, for example, binding domain-immunoglobulin fusion products encoded by the recombinant constructs of the present invention may occur, such as bacteria (for example, *E. coli*), yeast (for example, *Saccharomyces cerevisiae* and *Pichia pastoris*), insect cells, and mammalian cells, including *in vitro* and *in vivo* expression. Host organisms thus may include organisms for the
20 construction, propagation, expression or other steps in the production of the compositions provided herein. Hosts include subjects in which immune responses take place, as described herein. Presently preferred host organisms for production of constructs of the invention that produce glycosylated proteins are mammalian cells or other cells systems that permit the expression and recovery of glycosylated proteins. Other cell lines include inbred murine
25 strains and murine cell lines, and human cells and cell lines.

 A DNA construct encoding a desired construct of the invention, for example, a binding domain-immunoglobulin fusion protein, is introduced into a vector, for example, a plasmid, for expression in an appropriate host. In preferred embodiments, the host is a mammalian host, for example, a mammalian cell line. The sequence encoding the ligand or
30 nucleic acid binding domain is preferably codon-optimized for expression in the particular

host. Thus, for example, if a construct, for example, is a human binding domain-immunoglobulin fusion and is expressed in bacteria, the codons may be optimized for bacterial usage. For small coding regions, the gene can be synthesized as a single oligonucleotide. For larger proteins, splicing of multiple oligonucleotides, mutagenesis, or other techniques known to those in the art may be used. The sequences of nucleotides in plasmids or other vectors that are regulatory regions, such as promoters and operators, are operationally associated with one another for transcription. The sequence of nucleotides encoding a binding domain-immunoglobulin fusion protein may also include DNA encoding a secretion signal, whereby the resulting peptide is a precursor protein. The resulting processed protein may be recovered from the periplasmic space or the fermentation medium.

In preferred embodiments, the DNA plasmids may also include a transcription terminator sequence. As used herein, a "transcription terminator region" is a sequence that signals transcription termination. The entire transcription terminator may be obtained from a protein-encoding gene, which may be the same or different from the inserted binding domain-immunoglobulin fusion encoding gene or the source of the promoter. Transcription terminators are optional components of the expression systems herein, but are employed in preferred embodiments.

The plasmids or other vectors used herein include a promoter in operative association with the DNA encoding the protein or polypeptide of interest and are designed for expression of proteins in a suitable host as described above (*e.g.*, bacterial, murine, or human) depending upon the desired use of the plasmid (*e.g.*, administration of a vaccine containing binding domain-immunoglobulin fusion encoding sequences). Suitable promoters for expression of proteins and polypeptides herein are widely available and are well known in the art. Inducible promoters or constitutive promoters that are linked to regulatory regions are preferred. Such promoters include, for example, but are not limited to, the T7 phage promoter and other T7-like phage promoters, such as the T3, T5 and SP6 promoters, the *trp*, *lpp*, and *lac* promoters, such as the *lacUV5*, from *E. coli*; the P10 or polyhedrin gene promoter of baculovirus/insect cell expression systems (*see, e.g.*, U.S. Patent Nos. 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784) and inducible promoters from other eukaryotic expression systems. For expression of the proteins such

promoters are inserted in a plasmid in operative linkage with a control region such as the lac operon.

Preferred promoter regions are those that are inducible and functional in mammalian cells, for example. Examples of suitable inducible promoters and promoter regions for bacterial expression include, but are not limited to: the *E. coli* lac operator responsive to isopropyl β -D-thiogalactopyranoside (IPTG; see Nakamura *et al.*, 1979 *Cell* 18:1109-1117); the metallothionein promoter metal-regulatory-elements responsive to heavy-metal (*e.g.*, zinc) induction (see, *e.g.*, U.S. Patent No. 4,870,009); the phage T7lac promoter responsive to IPTG (see, *e.g.*, U.S. Patent No. 4,952,496; and Studier *et al.*, 1990 *Meth. Enzymol.* 185:60-89) and the TAC promoter. Depending on the expression host system to be used, plasmids may optionally include a selectable marker gene or genes that are functional in the host. Thus, for example, a selectable marker gene includes any gene that confers a phenotype on bacteria that allows transformed bacterial cells to be identified and selectively grown from among a vast majority of untransformed cells. Suitable selectable marker genes for bacterial hosts, for example, include the ampicillin resistance gene (Amp^r), tetracycline resistance gene (Tc^r) and the kanamycin resistance gene (Kan^r). The kanamycin resistance gene is presently preferred for bacterial expression.

In various expression systems, plasmids or other vectors may also include DNA encoding a signal for secretion of the operably linked protein. Secretion signals suitable for use are widely available and are well known in the art. Prokaryotic and eukaryotic secretion signals functional in *E. coli* may be employed. Depending on the expression systems, presently preferred secretion signals may include, but are not limited to, those encoded by the following *E. coli* genes: ompA, ompT, ompF, ompC, beta-lactamase, and alkaline phosphatase, and the like (von Heijne, *J. Mol. Biol.* 184:99-105, 1985). In addition, the bacterial pelB gene secretion signal (Lei *et al.*, *J. Bacteriol.* 169:4379, 1987), the phoA secretion signal, and the cek2 functional in insect cell may be employed. The most preferred secretion signal for certain expression systems is the *E. coli* ompA secretion signal. Other prokaryotic and eukaryotic secretion signals known to those of skill in the art may also be employed (*see, e.g.*, von Heijne, *J. Mol. Biol.* 184:99-105, 1985). Using the methods described herein, one of skill in the art can substitute secretion

signals that are functional in either yeast, insect or mammalian cells to secrete proteins from those cells.

Preferred plasmids for transformation of *E. coli* cells include the pET expression vectors (*e.g.*, pET-11a, pET-12a-c, pET-15b; *see* U.S. Patent No. 4,952,496; available from Novagen, Madison, WI.). Other preferred plasmids include the pKK plasmids, particularly pKK 223-3, which contains the tac promoter (Brosius *et al.*, 1984 *Proc. Natl. Acad. Sci.* 81:6929; Ausubel *et al.*, *Current Protocols in Molecular Biology*; U.S. Patent Nos. 5,122,463, 5,173,403, 5,187,153, 5,204,254, 5,212,058, 5,212,286, 5,215,907, 5,220,013, 5,223,483, and 5,229,279). Plasmid pKK has been modified by replacement of the ampicillin resistance gene with a kanamycin resistance gene. (Available from Pharmacia; obtained from pUC4K, *see, e.g.*, Vieira *et al.* (1982 *Gene* 19:259-268; and U.S. Patent No. 4,719,179.) Baculovirus vectors, such as pBlueBac (also called pJVETL and derivatives thereof), particularly pBlueBac III (*see, e.g.*, U.S. Patent Nos. 5,278,050, 5,244,805, 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784; available from Invitrogen, San Diego) may also be used for expression of the polypeptides in insect cells. Other plasmids include the pIN-IIIompA plasmids (*see* U.S. Patent No. 4,575,013; *see also* Duffaud *et al.*, *Meth. Enz.* 153:492-507, 1987), such as pIN-IIIompA2.

Preferably, if one or more DNA molecules is replicated in bacterial cells, the preferred host is *E. coli*. The preferred DNA molecule is such a system also includes a bacterial origin of replication, to ensure the maintenance of the DNA molecule from generation to generation of the bacteria. In this way, large quantities of the DNA molecule can be produced by replication in bacteria. In such expression systems, preferred bacterial origins of replication include, but are not limited to, the fl-ori and col E1 origins of replication. Preferred hosts for such systems contain chromosomal copies of DNA encoding T7 RNA polymerase operably linked to an inducible promoter, such as the lacUV promoter (*see* U.S. Patent No. 4,952,496). Such hosts include, but are not limited to, lysogens *E. coli* strains HMS174(DE3)pLysS, BL21(DE3)pLysS, HMS174(DE3) and BL21(DE3). Strain BL21(DE3) is preferred. The pLys strains provide low levels of T7 lysozyme, a natural inhibitor of T7 RNA polymerase.

The DNA molecules provided may also contain a gene coding for a repressor protein. The repressor protein is capable of repressing the transcription of a promoter that contains sequences of nucleotides to which the repressor protein binds. The promoter can be derepressed by altering the physiological conditions of the cell. For example, the alteration
5 can be accomplished by adding to the growth medium a molecule that inhibits the ability to interact with the operator or with regulatory proteins or other regions of the DNA or by altering the temperature of the growth media. Preferred repressor proteins include, but are not limited to the *E. coli* lacI repressor responsive to IPTG induction, the temperature sensitive λ cI857 repressor, and the like. The *E. coli* lacI repressor is preferred.

10 In general, recombinant constructs of the subject invention will also contain elements necessary for transcription and translation. In particular, such elements are preferred where the recombinant expression construct containing nucleic acid sequences encoding binding domain-immunoglobulin fusion proteins is intended for expression in a host cell or organism. In certain embodiments of the present invention, cell type preferred
15 or cell type specific expression of a cell binding domain-immunoglobulin fusion encoding gene may be achieved by placing the gene under regulation of a promoter. The choice of the promoter will depend upon the cell type to be transformed and the degree or type of control desired. Promoters can be constitutive or active and may further be cell type specific, tissue specific, individual cell specific, event specific, temporally specific or inducible. Cell-type
20 specific promoters and event type specific promoters are preferred. Examples of constitutive or nonspecific promoters include the SV40 early promoter (U.S. Patent No. 5,118,627), the SV40 late promoter (U.S. Patent No. 5,118,627), CMV early gene promoter (U.S. Patent No. 5,168,062), and adenovirus promoter. In addition to viral promoters, cellular promoters are also amenable within the context of this invention. In particular,
25 cellular promoters for the so-called housekeeping genes are useful. Viral promoters are preferred, because generally they are stronger promoters than cellular promoters. Promoter regions have been identified in the genes of many eukaryotes including higher eukaryotes, such that suitable promoters for use in a particular host can be readily selected by those skilled in the art.

Inducible promoters may also be used. These promoters include MMTV LTR (PCT WO 91/13160), inducible by dexamethasone; metallothionein promoter, inducible by heavy metals; and promoters with cAMP response elements, inducible by cAMP. By using an inducible promoter, the nucleic acid sequence encoding a binding domain-immunoglobulin fusion protein may be delivered to a cell by the subject invention expression construct and will remain quiescent until the addition of the inducer. This allows further control on the timing of production of the gene product.

Event-type specific promoters are active or up-regulated only upon the occurrence of an event, such as tumorigenicity or viral infection. The HIV LTR is a well known example of an event-specific promoter. The promoter is inactive unless the *tat* gene product is present, which occurs upon viral infection. Some event-type promoters are also tissue-specific.

Additionally, promoters that are coordinately regulated with a particular cellular gene may be used. For example, promoters of genes that are coordinately expressed may be used when expression of a particular binding construct of the invention, for example, a binding domain-immunoglobulin fusion protein-encoding gene is desired in concert with expression of one or more additional endogenous or exogenously introduced genes. This type of promoter is especially useful when one knows the pattern of gene expression relevant to induction of an immune response in a particular tissue of the immune system, so that specific immunocompetent cells within that tissue may be activated or otherwise recruited to participate in the immune response.

In addition to the promoter, repressor sequences, negative regulators, or tissue-specific silencers may be inserted to reduce non-specific expression of binding domain-immunoglobulin fusion protein encoding genes in certain situations, such as, for example, a host that is transiently immunocompromised as part of a therapeutic strategy. Multiple repressor elements may be inserted in the promoter region. Repression of transcription is independent on the orientation of repressor elements or distance from the promoter. One type of repressor sequence is an insulator sequence. Such sequences inhibit transcription (Dunaway *et al.*, 1997 *Mol Cell Biol* 17: 182-9; Gdula *et al.*, 1996 *Proc Natl Acad Sci USA* 93:9378-83, Chan *et al.*, 1996 *J Virol* 70: 5312-28; Scott and Geyer, 1995

EMBO J 14:6258-67; Kalos and Fournier, 1995 *Mol Cell Biol* 15:198-207; Chung *et al.*, 1993 *Cell* 74: 505-14) and will silence undesired background transcription.

Repressor elements have also been identified in the promoter regions of the genes for type II (cartilage) collagen, choline acetyltransferase, albumin (Hu *et al.*, 1992 *J. Cell Growth Differ.* 3(9):577-588), phosphoglycerate kinase (PGK-2) (Misuno *et al.*, 1992 *Gene* 119(2):293-297), and in the 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase gene. (Lemaigre *et al.*, *Mol. Cell Biol.* 11(2):1099-1106). Furthermore, the negative regulatory element Tse-1 has been identified in a number of liver specific genes, and has been shown to block cAMP response element(CRE)-mediated induction of gene activation in hepatocytes. (Boshart *et al.*, 1990 *Cell* 61(5):905-916,).

In preferred embodiments, elements that increase the expression of the desired product are incorporated into the construct. Such elements include internal ribosome binding sites (IRES; Wang and Siddiqui, 1995 *Curr. Top. Microbiol. Immunol* 203:99; Ehrenfeld and Semler, 1995 *Curr. Top. Microbiol. Immunol.* 203:65; Rees *et al.*, 1996 *Biotechniques* 20:102; Sugimoto *et al.*, 1994 *Biotechnology* 12:694). IRES increase translation efficiency. As well, other sequences may enhance expression. For some genes, sequences especially at the 5' end inhibit transcription and/or translation. These sequences are usually palindromes that can form hairpin structures. Any such sequences in the nucleic acid to be delivered are generally deleted. Expression levels of the transcript or translated product are assayed to confirm or ascertain which sequences affect expression. Transcript levels may be assayed by any known method, including Northern blot hybridization, RNase probe protection and the like. Protein levels may be assayed by any known method, including ELISA, western blot, immunocytochemistry or other well known techniques.

Other elements may be incorporated into the constructs of the invention, for example, into binding domain-immunoglobulin fusion protein encoding constructs of the present invention. In preferred embodiments, the construct includes a transcription terminator sequence, including a polyadenylation sequence, splice donor and acceptor sites, and an enhancer. Other elements useful for expression and maintenance of the construct in mammalian cells or other eukaryotic cells may also be incorporated (*e.g.*, origin of replication). Because the constructs are conveniently produced in bacterial cells, elements

that are necessary for, or that enhance, propagation in bacteria are incorporated. Such elements include an origin of replication, a selectable marker and the like.

As provided herein, an additional level of controlling the expression of nucleic acids encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, delivered to cells for gene therapy, for example, may be provided by simultaneously delivering two or more differentially regulated nucleic acid constructs. The use of such a multiple nucleic acid construct approach may permit coordinated regulation of an immune response such as, for example, spatiotemporal coordination that depends on the cell type and/or presence of another expressed encoded component. Those familiar with the art will appreciate that multiple levels of regulated gene expression may be achieved in a similar manner by selection of suitable regulatory sequences, including but not limited to promoters, enhancers and other well known gene regulatory elements.

The present invention also relates to vectors, and to constructs prepared from known vectors that include nucleic acids of the present invention, and in particular to “recombinant expression constructs”, including any of various known constructs, including delivery constructs, useful for gene therapy, that include any nucleic acids encoding, for example, binding domain-immunoglobulin fusion proteins and polypeptides according to the invention as provided herein; to host cells which are genetically engineered with vectors and/or other constructs of the invention and to methods of administering expression or other constructs comprising nucleic acid sequences encoding, for example, binding domain-immunoglobulin fusion polypeptides and fusion proteins of the invention, or fragments or variants thereof, by recombinant techniques.

Various constructs of the invention, including for example, binding domain-immunoglobulin fusion proteins, can be expressed in virtually any host cell, including *in vivo* host cells in the case of use for gene therapy, under the control of appropriate promoters, depending on the nature of the construct (*e.g.*, type of promoter, as described above), and on the nature of the desired host cell (*e.g.*, whether postmitotic terminally differentiated or actively dividing; *e.g.*, whether the expression construct occurs in host cell as an episome or is integrated into host cell genome).

Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described, for example, by Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, NY, (1989); as noted herein, in particularly preferred embodiments of the invention, recombinant expression is conducted in

5 mammalian cells that have been transfected or transformed with the subject invention recombinant expression construct. See also, for example, Machida, CA., "Viral Vectors for Gene Therapy: Methods and Protocols"; Wolff, JA, "Gene Therapeutics: Methods and Applications of Direct Gene Transfer" (Birkhauser 1994); Stein, U and Walther, W (eds.P, "Gene Therapy of Cancer: Methods and Protocols" (Humana Press 2000); Robbins, PD

10 (ed.), "Gene Therapy Protocols" (Humana Press 1997); Morgan, JR (ed.), "Gene Therapy Protocols" (Humana Press 2002); Meager, A (ed.), "Gene Therapy Technologies, Applications and Regulations: From Laboratory to Clinic" (John Wiley & Sons Inc. 1999); MacHida, CA and Constant, JG, "Viral Vectors for Gene Therapy: Methods and Protocols" (Humana Press 2002); "New Methods Of Gene Therapy For Genetic Metabolic Diseases

15 NIH Guide," Volume 22, Number 35, October 1, 1993. See also recent U.S. patents relating to gene therapy, including vaccines, which include U.S. Pat. Nos. 6,384,210 ("Solvent for biopolymer synthesis, solvent microdroplets and methods of use"); 6,384,203 ("Family of immunoregulators designated leukocyte immunoglobulin-like receptors (LIR)"); 6,384,202 ("Cell-specific active compounds regulated by the cell cycle"); 6,384,018 ("Polynucleotide

20 tuberculosis vaccine"); 6,383,814 ("Cationic amphiphiles for intracellular delivery of therapeutic molecules"); 6,383,811 ("Polyampholytes for delivering polyions to a cell"); 6,383,795 ("Efficient purification of adenovirus"); 6,383,794 ("Methods of producing high titer recombinant adeno-associated virus"); 6,383,785 ("Self-enhancing, pharmacologically controllable expression systems"); 6,383,753 ("Yeast mammalian regulators of cell

25 proliferation"); 6,383,746 ("Functional promoter for CCR5"); 6,383,743 ("Method for serial analysis of gene expression"); 6,383,738 ("Herpes simplex virus ORF P is a repressor of viral protein synthesis"); 6,383,737 ("Human oxalyl-CoA Decarboxylase"); 6,383,733 ("Methods of screening for pharmacologically active compounds for the treatment of tumour diseases"); 6,383,522 ("Toxicity reduced composition containing an anti-neoplastic agent

30 and a shark cartilage extract"); 6,383,512 ("Vesicular complexes and methods of making and

using the same"); 6,383,481 ("Method for transplantation of hemopoietic stem cells"); 6,383,478 ("Polymeric encapsulation system promoting angiogenesis"); 6,383,138 ("Method for transdermal sampling of analytes"); 6,380,382 ("Gene encoding a protein having diagnostic, preventive, therapeutic, and other uses"); 6,380,371 ("Endoglycan: a novel protein having selectin ligand and chemokine presentation activity"); 6,380,369 ("Human DNA mismatch repair proteins"); 6,380,362 ("Polynucleotides, polypeptides expressed by the polynucleotides and methods for their use"); 6,380,170 ("Nucleic acid construct for the cell cycle regulated expression of structural genes"); 6,380,169 ("Metal complex containing oligonucleoside cleavage compounds and therapies"); 6,379,967 ("Herpesvirus saimiri as viral vector"); 6,379,966 ("Intravascular delivery of non-viral nucleic acid protease proteins, and uses thereof").

Typically, for example, expression constructs are derived from plasmid vectors. One preferred construct is a modified pNASS vector (Clontech, Palo Alto, CA), which has nucleic acid sequences encoding an ampicillin resistance gene, a polyadenylation signal and a T7 promoter site. Other suitable mammalian expression vectors are well known (*see, e.g.,* Ausubel *et al.*, 1995; Sambrook *et al., supra*; *see also, e.g.,* catalogues from Invitrogen, San Diego, CA; Novagen, Madison, WI; Pharmacia, Piscataway, NJ; and others). Presently preferred constructs may be prepared that include a dihydrofolate reductase (DHFR) encoding sequence under suitable regulatory control, for promoting enhanced production levels of the binding domain-immunoglobulin fusion protein, which levels result from gene amplification following application of an appropriate selection agent (*e.g.,* methotrexate).

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, as described above. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences. Thus, for example, the binding domain-immunoglobulin fusion protein encoding nucleic acids as provided herein may be included in any one of a variety of expression vector constructs as a recombinant expression construct for expressing a binding domain-immunoglobulin fusion polypeptide in a host cell.

In certain preferred embodiments the constructs are included in formulations that are administered *in vivo*. Such vectors and constructs include chromosomal, nonchromosomal and synthetic DNA sequences, *e.g.*, derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA, such as vaccinia, adenovirus, fowl pox virus, and pseudorabies, or replication deficient retroviruses as described below. However, any other vector may be used for preparation of a recombinant expression construct, and in preferred embodiments such a vector will be replicable and viable in the host.

The appropriate DNA sequence(s) may be inserted into a vector, for example, by a variety of procedures. In general, a DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described, for example, in Ausubel *et al.* (1993 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, MA); Sambrook *et al.* (1989 *Molecular Cloning*, Second Ed., Cold Spring Harbor Laboratory, Plainview, NY); Maniatis *et al.* (1982 *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, NY); Glover (Ed.) (1985 *DNA Cloning Vol. I and II*, IRL Press, Oxford, UK); Hames and Higgins (Eds.), (1985 *Nucleic Acid Hybridization*, IRL Press, Oxford, UK); and elsewhere.

The DNA sequence in the expression vector is operatively linked to at least one appropriate expression control sequence(s) (*e.g.*, a constitutive promoter or a regulated promoter) to direct mRNA synthesis. Representative examples of such expression control sequences include promoters of eukaryotic cells or their viruses, as described above. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art, and preparation of certain particularly preferred

recombinant expression constructs comprising at least one promoter or regulated promoter operably linked to a nucleic acid encoding an binding domain-immunoglobulin fusion polypeptide is described herein.

Transcription of the DNA encoding proteins and polypeptides included within the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are *cis*-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Gene therapy is the use of genetic material to treat disease. It comprises strategies to replace defective genes or add new genes to cells and/or tissues, and is being developed for application in the treatment of cancer, the correction of metabolic disorders and in the field of immunotherapy. Gene therapies of the invention include the use of various constructs of the invention, with or without a separate carrier or delivery vehicle or constructs, for treatment of the diseases, disorders, and/or conditions noted herein. Such constructs may also be used as vaccines for treatment or prevention of the diseases, disorders, and/or conditions noted herein. DNA vaccines, for example, make use of polynucleotides encoding immunogenic protein and nucleic acid determinants to stimulate the immune system against pathogens or tumor cells. Such strategies can stimulate either acquired or innate immunity or can involve the modification of immune function through cytokine expression. *In vivo* gene therapy involves the direct injection of genetic material into a patient or animal model of human disease. Vaccines and immune modulation are systemic therapies. With tissue-specific *in vivo* therapies, such as those that aim to treat cancer, localized gene delivery and/or expression/targeting systems are preferred. Diverse gene therapy vectors have been designed to target specific tissues, and procedures have been developed to physically target specific tissues, for example, using catheter-based technologies, all of which are contemplated herein. *Ex vivo* approaches to gene therapy are also contemplated herein and involve the removal, genetic modification, expansion and re-administration of a patient's own cells. Examples include bone marrow transplantation for

cancer treatment or the genetic modification of lymphoid progenitor cells. *Ex vivo* gene therapy is preferably applied to the treatment of cells that are easily accessible and can survive in culture during the gene transfer process (such as blood or skin cells).

Useful gene therapy vectors include adenoviral vectors, lentiviral vectors, Adeno-associated virus (AAV) vectors, Herpes Simplex Virus (Hsv) vectors, and retroviral vectors. Gene therapies may also be carried out using "naked DNA," liposome-based delivery, lipid-based delivery (including DNA attached to positively charged lipids), and electroporation.

As provided herein, in certain embodiments, including but not limited to gene therapy embodiments, the vector may be a viral vector such as, for example, a retroviral vector. Miller *et al.*, 1989 *BioTechniques* 7:980; Coffin and Varmus, 1996 *Retroviruses*, Cold Spring Harbor Laboratory Press, NY. For example, retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

Retroviruses are RNA viruses which can replicate and integrate into the genome of a host cell via a DNA intermediate. This DNA intermediate, or provirus, may be stably integrated into the host cell DNA. According to certain embodiments of the present invention, an expression construct may comprise a retrovirus into which a foreign gene that encodes a foreign protein is incorporated in place of normal retroviral RNA. When retroviral RNA enters a host cell coincident with infection, the foreign gene is also introduced into the cell, and may then be integrated into host cell DNA as if it were part of the retroviral genome. Expression of this foreign gene within the host results in expression of the foreign protein.

Most retroviral vector systems which have been developed for gene therapy are based on murine retroviruses. Such retroviruses exist in two forms, as free viral particles referred to as virions, or as proviruses integrated into host cell DNA. The virion form of the virus contains the structural and enzymatic proteins of the retrovirus (including the enzyme reverse transcriptase), two RNA copies of the viral genome, and portions of the source cell

plasma membrane containing viral envelope glycoprotein. The retroviral genome is organized into four main regions: the Long Terminal Repeat (LTR), which contains *cis*-acting elements necessary for the initiation and termination of transcription and is situated both 5' and 3' of the coding genes, and the three coding genes *gag*, *pol*, and *env*. These
5 three genes *gag*, *pol*, and *env* encode, respectively, internal viral structures, enzymatic proteins (such as integrase), and the envelope glycoprotein (designated gp70 and p15e) which confers infectivity and host range specificity of the virus, as well as the "R" peptide of undetermined function.

Separate packaging cell lines and vector producing cell lines have been
10 developed because of safety concerns regarding the uses of retroviruses, including their use in expression constructs as provided by the present invention. Briefly, this methodology employs the use of two components, a retroviral vector and a packaging cell line (PCL). The retroviral vector contains long terminal repeats (LTRs), the foreign DNA to be transferred and a packaging sequence (*y*). This retroviral vector will not reproduce by itself
15 because the genes which encode structural and envelope proteins are not included within the vector genome. The PCL contains genes encoding the *gag*, *pol*, and *env* proteins, but does not contain the packaging signal "*y*". Thus, a PCL can only form empty virion particles by itself. Within this general method, the retroviral vector is introduced into the PCL, thereby creating a vector-producing cell line (VCL). This VCL manufactures virion particles
20 containing only the retroviral vector's (foreign) genome, and therefore has previously been considered to be a safe retrovirus vector for therapeutic use.

"Retroviral vector construct" refers to an assembly which is, within preferred embodiments of the invention, capable of directing the expression of a sequence(s) or gene(s) of interest, such as binding domain-immunoglobulin fusion encoding nucleic acid
25 sequences. Briefly, the retroviral vector construct must include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis and a 3' LTR. A wide variety of heterologous sequences may be included within the vector construct, including for example, sequences which encode a protein (*e.g.*, cytotoxic protein, disease-associated antigen, immune accessory molecule, or replacement gene), or which are useful as a
30 molecule itself (*e.g.*, as a ribozyme or antisense sequence).

Retroviral vector constructs of the present invention may be readily constructed from a wide variety of retroviruses, including for example, B, C, and D type retroviruses as well as spumaviruses and lentiviruses (see, *e.g.*, RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985). Such retroviruses may be readily
5 obtained from depositories or collections such as the American Type Culture Collection ("ATCC"; Rockville, Maryland), or isolated from known sources using commonly available techniques. Any of the above retroviruses may be readily utilized in order to assemble or construct retroviral vector constructs, packaging cells, or producer cells of the present invention given the disclosure provided herein, and standard recombinant techniques (*e.g.*,
10 Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, 1989; Kunkle, 1985 *PNAS* 82:488).

Suitable promoters for use in viral vectors generally may include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, *et al.*, 1989 *Biotechniques* 7:980-990, or any other promoter
15 (*e.g.*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β -actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein, and may be from among either
20 regulated promoters or promoters as described above.

As described above, the retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ψ -2, ψ -AM, PA12, T19-14X, VT-19-17-H2, ψ CRE, ψ CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in
25 Miller, *Human Gene Therapy*, 1:5-14 (1990). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO_4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the binding domain-immunoglobulin fusion polypeptides or fusion proteins. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will
5 express the nucleic acid sequence(s) encoding the binding domain-immunoglobulin fusion polypeptide or fusion protein. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, circulating peripheral blood mononuclear and polymorphonuclear cells including myelomonocytic cells, lymphocytes, myoblasts, tissue macrophages, dendritic cells, Kupffer
10 cells, lymphoid and reticuloendothelia cells of the lymph nodes and spleen, keratinocytes, endothelial cells, and bronchial epithelial cells.

As another example of an embodiment of the invention in which a viral vector is used to prepare, for example, a recombinant binding domain-immunoglobulin fusion expression construct, in one preferred embodiment, host cells transduced by a
15 recombinant viral construct directing the expression of binding domain-immunoglobulin fusion polypeptides or fusion proteins may produce viral particles containing expressed binding domain-immunoglobulin fusion polypeptides or fusion proteins that are derived from portions of a host cell membrane incorporated by the viral particles during viral budding.

20 In another aspect, the present invention relates to host cells containing the herein described nucleic acid constructs, such as, for example, recombinant binding domain-immunoglobulin fusion expression constructs. Host cells are genetically engineered (transduced, transformed or transfected) with the vectors and/or expression constructs of this invention which may be, for example, a cloning vector, a shuttle vector, or an expression
25 construct. The vector or construct may be, for example, in the form of a plasmid, a viral particle, a phage, *etc.* The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying particular genes such as genes encoding binding domain-immunoglobulin fusion polypeptides or binding domain-immunoglobulin fusion fusion proteins. The culture

conditions for particular host cells selected for expression, such as temperature, pH and the like, will be readily apparent to the ordinarily skilled artisan.

The host cell for production or expression of a construct of the invention, for example, can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Representative examples of appropriate host cells according to the present invention include, but need not be limited to, bacterial cells, such as *E. coli*, *Streptomyces*, *Salmonella typhimurium*; fungal cells, such as yeast; insect cells, such as *Drosophila S2* and *Spodoptera Sf9*; animal cells, such as CHO, COS or 293 cells; adenoviruses; plant cells, or any suitable cell already adapted to *in vitro* propagation or so established *de novo*. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, 1981 *Cell* 23:175, and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences, for example as described herein regarding the preparation of binding domain-immunoglobulin fusion expression constructs. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Introduction of the construct into the host cell can be effected by a variety of methods with which those skilled in the art will be familiar, including but not limited to, for example, calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis *et al.*, 1986 *Basic Methods in Molecular Biology*).

The present invention constructs, for example, binding domain-immunoglobulin fusion proteins, or compositions comprising one or more polynucleotides encoding same as described herein (for example, to be administered under conditions and

for a time sufficient to permit expression of a binding domain-immunoglobulin fusion protein in a host cell *in vivo* or *in vitro*, for gene therapy, for example, among other things), may be formulated into pharmaceutical compositions for administration according to well known methodologies. Pharmaceutical compositions generally comprise one or more
5 recombinant expression constructs, and/or expression products of such constructs, in combination with a pharmaceutically acceptable carrier, excipient or diluent. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. For nucleic acid-based formulations, or for formulations comprising expression products of the subject invention recombinant constructs, about 0.01 µg/kg to about 100 mg/kg body weight will be
10 administered, for example, typically by the intradermal, subcutaneous, intramuscular or intravenous route, or by other routes. A preferred dosage, for example, is about 1 µg/kg to about 1 mg/kg, with about 5 µg/kg to about 200 µg/kg particularly preferred. It will be evident to those skilled in the art that the number and frequency of administration will be dependent upon the response of the host. "Pharmaceutically acceptable carriers" for
15 therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remingtons Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of
20 *p*-hydroxybenzoic acid may be added as preservatives. *Id.* at 1449. In addition, antioxidants and suspending agents may be used. *Id.*

"Pharmaceutically acceptable salt" refers to salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid (acid addition salts) or an organic or inorganic base (base addition salts). The
25 compounds of the present invention may be used in either the free base or salt forms, with both forms being considered as being within the scope of the present invention.

The pharmaceutical compositions that contain one or more nucleic acid constructs of the invention, for example, binding domain-immunoglobulin fusion protein encoding constructs (or their expressed products) may be in any form which allows for the
30 composition to be administered to a patient. For example, the composition may be in the

form of a solid, liquid or gas (aerosol). Typical routes of administration include, without limitation, oral, topical, parenteral (*e.g.*, sublingually or buccally), sublingual, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal, intracavernous, intrathecal, intrameatal, intraurethral injection or infusion techniques. The pharmaceutical composition is formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of one or more compounds of the invention in aerosol form may hold a plurality of dosage units.

For oral administration, an excipient and/or binder may be present. Examples are sucrose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose and ethyl cellulose. Coloring and/or flavoring agents may be present. A coating shell may be employed.

The composition may be in the form of a liquid, *e.g.*, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred compositions contain, in addition to one or more binding domain-immunoglobulin fusion construct or expressed product, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

A liquid pharmaceutical composition as used herein, whether in the form of a solution, suspension or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and

agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

5 It may also be desirable to include other components in the preparation, such as delivery vehicles including but not limited to aluminum salts, water-in-oil emulsions, biodegradable oil vehicles, oil-in-water emulsions, biodegradable microcapsules, and liposomes. Examples of immunostimulatory substances (adjuvants) for use in such vehicles include N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), lipopoly-saccharides (LPS),
10 glucan, IL-12, GM-CSF, gamma interferon and IL-15.

 While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration and whether a sustained release is desired. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises
15 water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable
20 microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109. In this regard, it is preferable that the microsphere be larger than approximately 25 microns.

 Pharmaceutical compositions may also contain diluents such as buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans,
25 chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (*e.g.*, sucrose) as diluents.

 As described above, the subject invention includes compositions capable of
30 delivering nucleic acid molecules encoding binding domain-immunoglobulin fusion

proteins. Such compositions include recombinant viral vectors (e.g., retroviruses (see WO 90/07936, WO 91/02805, WO 93/25234, WO 93/25698, and WO 94/03622), adenovirus (see Berkner, 1988 *Biotechniques* 6:616-627; Li *et al.*, 1993 *Hum. Gene Ther.* 4:403-409; Vincent *et al.*, *Nat. Genet.* 5:130-134; and Kolls *et al.*, 1994 *Proc. Natl. Acad. Sci. USA* 91:215-219), pox virus (see U.S. Patent No. 4,769,330; U.S. Patent No. 5,017,487; and WO 89/01973)), recombinant expression construct nucleic acid molecules complexed to a polycationic molecule (see WO 93/03709), and nucleic acids associated with liposomes (see Wang *et al.*, 1987 *Proc. Natl. Acad. Sci. USA* 84:7851). In certain embodiments, the DNA may be linked to killed or inactivated adenovirus (see Curiel *et al.*, 1992 *Hum. Gene Ther.* 3:147-154; Cotton *et al.*, 1992 *Proc. Natl. Acad. Sci. USA* 89:6094). Other suitable compositions include DNA-ligand (see Wu *et al.*, 1989 *J. Biol. Chem.* 264:16985-16987) and lipid-DNA combinations (see Felgner *et al.*, 1989 *Proc. Natl. Acad. Sci. USA* 84:7413-7417).

In addition to direct *in vivo* procedures, *ex vivo* procedures may be used in which cells are removed from a host, modified, and placed into the same or another host animal. It will be evident that one can utilize any of the compositions noted above for introduction of constructs of the invention, for example, binding domain-immunoglobulin fusion proteins or of binding domain-immunoglobulin fusion protein encoding nucleic acid molecules into tissue cells in an *ex vivo* context. Protocols for viral, physical and chemical methods of uptake are well known in the art.

Accordingly, the present invention is useful for treating a patient having a B cell disorder or a malignant condition, or for treating a cell culture derived from such a patient. As used herein, the term "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with cancer or a malignant condition, such as B cell lymphoma, or may be normal (*i.e.*, free of detectable disease and infection). A "cell culture" includes any preparation amenable to *ex vivo* treatment, for example a preparation containing immunocompetent cells or isolated cells of the immune system (including, but not limited to, T cells, macrophages, monocytes, B cells and dendritic cells). Such cells may be isolated by any of a variety of techniques well known to those of ordinary skill in the art (e.g., Ficoll-hypaque density centrifugation). The cells may (but need not) have been

isolated from a patient afflicted with a B cell disorder or a malignant condition, and may be reintroduced into a patient after treatment.

A liquid composition intended for either parenteral or oral administration should contain an amount of a construct of the invention, for example, a binding domain-immunoglobulin fusion protein encoding construct or expressed product, such that a suitable dosage will be obtained. Typically, this amount is at least 0.01 wt% of a binding domain-immunoglobulin fusion construct or expressed product in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Preferred oral compositions contain between about 4% and about 50% of binding domain-immunoglobulin fusion construct or expressed product(s). Preferred compositions and preparations are prepared so that, for example, a parenteral dosage unit contains between 0.01 to 1% by weight of active compound.

The pharmaceutical composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, beeswax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. Topical formulations may contain a concentration of a construct of the invention, for example, a binding domain-immunoglobulin fusion construct or expressed product, of from about 0.1 to about 10% w/v (weight per unit volume).

The composition may be intended for rectal administration, in the form, *e.g.*, of a suppository which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

In the methods of the invention, a construct of the invention, for example, a binding domain-immunoglobulin fusion encoding constructs or expressed product(s), may be administered through use of insert(s), bead(s), timed-release formulation(s), patch(es) or fast-release formulation(s).

Constructs of the invention, for example, antigen-binding constructs of the invention, may be administered or co-administered to an animal or patient in combination with, or at the same or about the same time, as other compounds. In one aspect, one or more constructs, including for example one or more antigen-binding constructs, are administered to an animal or patient in conjunction with one or more chemotherapeutic compounds such as alkylating agents, nucleoside analogues, and the like. The administration or co-administration of one or more constructs, including one or more antigen-binding constructs, of the invention and one or more chemotherapeutic agents can be used for the treatment of tumors or cancer in an animal or patient. Exemplary cancers include, but are not limited to, head and neck cancer, breast cancer, colorectal cancer, gastric cancer, hepatic cancer, bladder cancer, cervical cancer, endometrial cancer, lung cancer (non-small cell), ovarian cancer, pancreatic cancer, prostate cancer; choriocarcinoma (lung cancer); hairy cell leukemia, chronic lymphocytic leukemia, acute lymphocytic leukemia (breast & bladder), acute myelogenous leukemia, meningeal leukemia, chronic myelogenous leukemia, erythroleukemia. More commonly the cancers treated include non-Hodgkin's lymphoma (osteogenic sarcoma, adult soft tissue sarcoma), T-cell lymphoma, chronic lymphocytic leukaemia, slowly growing non-Hodgkin's lymphomas, Hodgkin's lymphoma and ovarian cancer.

Examples of an alkylating agents that can be co-administered with one or more constructs, including one or more antigen-binding constructs, of the invention include mechlorethamine, chlorambucil, ifosfamide, melphalan, busulfan, carmustine, lomustine, procarbazine, dacardazine, cisplatin, carboplatin, mitomycin C, cyclophosphamide, isosfamide, , hexamethylmelamine, thiotepa, , and dacarbazine, and analogues thereof. See for example U.S. Pat. No. 3,046,301 describing the synthesis of chlorambucil, U.S. Pat. No. 3,732,340 describing the synthesis of ifosfamide, U.S. Pat. No. 3,018,302 for the synthesis of cyclophosphamide, U.S. Pat. No. 3,032,584 describing the synthesis of melphalan, and Braunwald et al., "Harrison's Principles of Internal Medicine," 15th Ed., McGraw-Hill, New York, NY, pp.536-544 (2001) for clinical aspects of cyclophosphamide, chlorambucil, melphalan, ifosfamide, procarbazine, hexamethylmelamine, cisplatin, and carboplatin. Examples of nucleoside analogues, include, but are not limited to, fludarabine pentostatin,

methotrexate, fluorouracil, fluorodeoxyuridine, CB3717, azacitidine, cytarabine, floxuridine, mercaptopurine, 6-thioguanine, , cladribine and analogues thereof. One example is the combination of constructs, including antigen-binding constructs, that bind CD20. This construct acts as a chemosensitising agent and works together with chemotherapeutic agents, such that less chemotherapeutic agents are necessary to achieve anti-tumor or anti-cancer effects. For example, U.S. Pat. No. 3,923,785 describing the synthesis of pentostatin, U.S. Pat. No. 4,080,325 describing the synthesis of methotrexate, U.S. Pat. No. 2,802,005 describing the synthesis of fluorouracil, and Braunwald et al., "Harrison's Principles of Internal Medicine," 15th Ed., McGraw-Hill, New York, NY, pp.536-544 (2001) for clinical aspects of methotrexate, 5-fluorouracil, cytosine arabinoside, 6-mercaptopurine, 6-thioguanine, and fludarabine phosphate.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered compounds that inhibit topoisomerase II or compounds that otherwise interact with nucleic acids in cells. Such compounds include, for example, doxorubicin, epirubicin, etoposide, teniposide, mitoxantrone, and analogues thereof. In one example, this combination is used in treatment to reduce tumor cell contamination of peripheral blood progenitor cells (PBSC) in conjunction with high-dose chemotherapy and autologous stem cell support (HDC-ASCT). See U.S. Patent 6,586,428 to Geroni *et al.*

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with therapeutic drugs. For example, Virulizin (Lorus Therapeutics), which is believed to stimulate the release of tumour necrosis factor, TNF-alpha, by tumour cells in vitro and stimulate activation of macrophage cells. This can be used in combination with one or more constructs, including one or more antigen-binding constructs, of the invention to increase cancer cell apoptosis and treat various types of cancers including Pancreatic Cancer, Malignant Melanoma, Kaposi's Sarcoma (KS), Lung Cancer, Breast Cancer, Uterine, Ovarian and Cervical Cancer. Another example is CpG 7909 (Coley Pharmaceutical Group), which is believed to activate NK cells and monocytes and enhance ADCC. This drug can be used in combination with cancer or tumor specific constructs, including antigen-

binding constructs, of the invention, such as an anti-CD20 construct, to treat non-Hodgkin's lymphoma and other cancers.

One or more constructs, including one or more antigen-binding constructs, of the invention can also be combined with angiogenesis inhibitors to increase anti-tumor effects. Angiogenesis is the growth of new blood vessels. This process allows tumors to grow and metastasize. Inhibiting angiogenesis can help prevent metastasis, and stop the spread of tumors cells. Angiogenesis inhibitors include, but are not limited to, angiostatin, endostatin, thrombospondin, platelet factor 4, Cartilage-derived inhibitor (CDI), retinoids, Interleukin-12, tissue inhibitor of metalloproteinase 1, 2 and 3 (TIMP-1, TIMP-2, and TIMP-3) and proteins that block the angiogenesis signaling cascade, such as anti-VEGF (Vascular Endothelial Growth Factor) and IFN-alpha. Angiogenesis inhibitors can be administered or co-administered with tumor specific constructs, including antigen-binding constructs capable of mediating, for example, ADCC and/or complement fixation or chemotherapy-conjugated antigen-binding of the invention to combat various types of cancers, for example, solid tumor cancers such as lung and breast cancer.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with disease modifying anti-rheumatic agents (DMAR agents) for the treatment of rheumatoid arthritis, psoriasis, ulcerative colitis, systemic lupus erythematosus (SLE), Crohn's disease, ankylosing spondylitis, and various inflammatory disease processes. In such treatment, the constructs, for example, antigen-binding constructs, of the invention are commonly administered in conjunction with compounds such as azathioprine, cyclosporin, gold, hydroxychloroquine, methotrexate, penicillamine, sulphasalazine, and the like.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with agents or compounds that counteract the biological effects of interleukin-1, including for example interleukin-1 inhibitors and interleukin-1 receptor antagonist. It is thought that interleukin-1 has a role in the generation of rheumatoid arthritis (RA), inflammation, and the destruction of joints. IL-1 inhibitors can also be used in conjunction with the constructs, including antigen-binding constructs, of the invention to treat arthritis, inflammatory bowel disease,

sepsis and septic shock, ischemic injury, reperfusion, ischemic brain injury such as cerebral palsy and multiple sclerosis. See U.S. Patent No. 6,159,460 to Thompson *et al.* In another aspect, for example, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered to an animal or patient in
5 conjunction with one or more glucocorticoids for example, methylprednisilone, dexamethasone, hydrocortisone, and the like. Glucocorticoids have been used to induce apoptosis and inhibit growth, independent of ADCC and CDC. These compounds can be combined with constructs, including antigen-binding constructs, of the invention capable of inducing apoptosis in cancer cells. In one example is the anti-CD20, and anti-CD40
10 antigen-binding constructs, which can be used to induce apoptosis in B-cells, are combined with glucocorticoids to treat B-cell non-Hodgkin's lymphoma (NHL).

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with p38 inhibitors or antagonists. The p38 mitogen-activated protein kinase pathway is involved in a
15 number of cellular processes instrumental to the development of rheumatoid arthritis. For example, the activation and infiltration of leukocytes as well as the production of inflammatory cytokines are p38-dependent processes.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention are administered or co-administered with compounds
20 that promote the differentiation and proliferation of B-cells. Cytokines such as \ interleukin-4 (IL-4) and interleukin-6 (IL-6), in addition to other biological activities, have been shown to stimulate antibody synthesis and secretion by activated B lymphocytes. In a particular aspect of the invention, constructs, including antigen-binding constructs, that recognize and bind CD20 are co-administered with one or more of interleukin-4 (IL-4) and
25 interleukin-6 (IL-6).

In another aspect one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with Interleukin-2 (IL-2). Interleukin 2 (IL-2) is a lymphokine that increases production of effector cells, such as CD4+ T-helper cells, CD8 cytotoxic cells, antibody producing B cells,
30 natural killer cells (NK), and monocytes/macrophages. IL-2 helps produce T-cells, which in

turn secrete more of the IL-2 (an "autocrine loop"). IL-2 can be used to augment antibody-dependent cell-mediated cytotoxicity (ADCC) and immunotherapies associated with constructs of the invention. In one example, an anti-CD20 construct of the invention and IL-2 are used to treat patients with relapsed or refractory follicular non-Hodgkin's lymphoma.

5 In another example IL-2 is administered or co-administered with HIV immunotherapies to help with T cell recovery.

In another aspect one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with Interleukin-12 (IL-12). IL-12 is known to enhance cytolytic T-cell responses, promote the development of helper T cells, enhance the activity of natural killer (NK) cells, and induces the secretion of IFN- γ in T and NK cells. IL-12 also increases many helper and effector cells that mediate apoptosis. In another aspect of the invention, one or more constructs, including one or more antigen-binding constructs, are administered or co-administered with IL-12 in the treatment of an animal or patient with a tumor or cancer. For example, a
10 construct, including an antigen-binding construct, of the invention that binds CD20 combined with IL-2 for the treatment of a patient with B-cell non-Hodgkin's lymphoma (NHL).
15

One or more constructs, including one or more antigen-binding constructs, of the invention can also be combined with immunomodulators to boost the efficacy of the antigen-binding constructs of the invention. Immunomodulators include, but are not limited to, Colony Stimulating Factors (CSF), Tumor necrosis Factors (TNF), and Interferons (IFN).
20

CSFs can include granulocyte-macrophage CSF (GM-CSF), granulocyte-CSF (G-CSF), and macrophage CSF (M-CSF). GM-CSF is thought to regulate the development of neutrophils, macrophages, monocytes and eosinophils. G-CSF has been shown to induce neutrophil production, and M-CSF production. M-CSF has been shown to stimulate macrophages and monocytes. The use of CSFs to treat neutropenia in cancer patients has been long established. In one example, constructs, including antigen-binding constructs, of the invention can be combined with GM-CSF, G-CSF or combinations thereof
25

in order to accelerate recovery from neutropenia in patients after bone marrow transplantation and chemotherapy. Neutrophils play a major role in fighting microbes such as bacterial, fungi and parasites. Patients with neutropenia are particularly susceptible to bacterial and wide spread fungal infections. In another example, a construct, including an antigen binding construct, of the invention can be combined with GM-CSF-treated neutrophils, monocytes and macrophages to increase activity against bacteria, fungi, etc, including the dreaded *Pneumocystis carinii*.

An example of an IFN is interferon alpha (IFN- α). IFN- α is made naturally by some types of white blood cell as part of the immune response when the body reacts to cancers or viral infections. It has two main modes of attack, interfering with growth and proliferation of cancer cells and it boosting the production of killer T cells and other cells that attack cancer cells. Interferon is also thought to facilitate cancer cells to put out chemical signals that make them better targets for the immune system, and has been used in recent years for several different types of cancer, particularly kidney cancer, melanoma, multiple myeloma, and some types of leukemia. It is also used to treat viral infections such as hepatitis. Interferon-alpha2a, for example, enhances ADCC and can be combined with one or more constructs, including antigen-binding constructs, of the invention to increase the efficiency of ADCC activity associated with the construct. In another example, one or more constructs, including one or more antigen-binding constructs of the invention are administered or co-administered to an animal or patient with interferon-gamma (IFN- γ), which has been show to increase the number of anti-CD20 antigens on B cells and bone marrow plasma cells (BMPC). This is particularly useful for the treatment of patients with multiple myelomas, which have a reduced expression of CD20 in their B cells and bone marrow plasma cells (BMPC). Accordingly, the treatment of multiple myeloma patients with constructs, including antigen-binding constructs of the invention, in particular constructs that bind CD20, may be usefully co-administered in conjunction with IFN- γ

TNF is a class of natural chemicals with anticancer properties. One example of a TNF is TNF- alpha. TNF-alpha has also been shown to have synergistic effects with IFN-gamma and IL-12. In another example, TNF can be administered or co-administered

with one or more tumor specific constructs, including one or more antigen-binding constructs, of the invention, and include chemotherapy-conjugated antigen binding constructs of the invention, together with IFN-gamma, IL-12 or various combinations thereof. TNF is also known to be an inflammatory regulation molecule. TNF-alpha
5 antibodies or antagonist(s) can be combined with anti-T cell constructs, including antigen-binding constructs, of the invention to treat patients with rheumatoid arthritis, psoriasis, ulcerative colitis, systemic lupus erythematosus (SLE), Crohn's disease, ankylosing spondylitis, and various inflammatory disease processes.

In another aspect, one or more constructs, including one or more antigen-
10 binding constructs, of the invention can be administered or co-administered with another antibody or antigen-binding construct of the invention. One example is a construct, for example, an antigen-binding construct of the invention capable of binding CD20 combined with a construct capable of binding CD22, CD19 or combinations thereof. This combination is effective as a treatment for indolent and aggressive forms of B-cell lymphomas, and acute
15 and chronic forms of lymphatic leukemias. See U.S. Patent 6,306,393 to Goldberg. In another example, constructs, including antigen-binding constructs, of the invention are co-administered with other constructs such as antigen-binding constructs of the invention that aid in mediating apoptosis. For example, a combination of one or more constructs, including one or more antigen-binding constructs of the invention capable of binding CD28,
20 CD3, CD20 or a combination thereof. The combination of anti-CD28 and CD3 provides a method for prolonged proliferation of T-cells. See U.S. Patent No. 6,352,694 to June et al.. This prolonged T-cell proliferation increases the efficiency immune dependent cytotoxicity, particularly those associated with anti-CD20.

In another aspect, constructs, including antigen-binding constructs, of the
25 invention can be administered or co-administered with one or more T-cell regulatory molecules. One example is a combination with interleukin-12 (IL-12). The IL-12 cytokine stimulates cell-mediated immunity, has angiostatic activity, and possesses significant anti-tumor effects in a variety of tumor models. IL-12 has also been shown to stimulate the production of interferon-gamma (IFN- γ). Accordingly, the treatment of multiple myeloma

patients with one or more constructs, including one or more antigen-binding constructs, of the invention, in particular those that bind CD20, is expected to be more efficacious when co-administered in conjunction with IL-12. In another example, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with a binding-domain construct of the invention other protein capable of binding CTLA-4 to enhance the anti-tumor immune response, by inhibiting the downregulation of T-cell activation.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be combined with gene therapies. In one example, a chemotherapy-conjugated construct of the invention is administered or co-administered with the Bcl-2 antisense oligonucleotide. Bcl-2 is associated with tumor resistance to anti-cancer therapies, and its believed to blocking chemotherapy-induced cell death. In another example one or more constructs, including one or more antigen-binding constructs, of the invention is administered or co-administered with an adenovirus for delivery of a "suicide gene." The adenovirus inserts the gene directly into the tumor cells, which makes these cells sensitive to an otherwise ineffective drug. Drug treatment then destroys the tumor cells, while leaving healthy cells untouched. However, once therapy is complete stray cancer cells that escaped therapy can reestablish and metastasize. Combining gene therapy with one or more constructs, including one or more antigen-binding constructs, will help kill stray cancer cells and minimize cancer reoccurrence.

A similar combination can be used with palliative (non-radical) operations to surgically remove tumors. In this example one or more constructs, including one or more antigen-binding constructs, of the invention can be administered before and after surgical extractions of tumors in order to increase the immune response and reduce the likelihood of reoccurrence by killing any cancer cells that were not removed during the surgery.

Another aspect combines a cancer or antigen vaccine and T-cell regulator molecules. For example, the binding portion, for example, an antigen-binding portion, of a construct can be specific for a cancer cell or antigen, or a protein fragment from a cancer cell or antigen. This can help mediate an immune response against a particular tumor or

antigen. Such constructs can be combined with T-cell regulators to increase the efficiency of the immune response.

In another example, one or more constructs, including one or more antigen-binding constructs, of the invention is administered or co-administered with retinoids. Retinoids include Vitamin A and its derivatives, which have the ability to stop cells from dividing and cause them to differentiate. Vitamin A is combined with an anti-cancer construct(s), including antigen-binding construct(s), of the invention to combat various forms of cancer.

The terms "binding construct" and "antigen-binding construct" as used herein may refer to, for example, engineered polypeptides, recombinant polypeptides, synthetic, semi-synthetic or other fusion proteins that are capable of binding a target, for example, an antigen. Antigen-binding constructs of the invention may be used in various applications, including those within the variety of uses to which antibodies or related immunoglobulin-type constructs may be put. Constructs, including antigen-binding constructs of the invention can be used in *in vivo* and *in vitro* experiments for therapeutic, diagnostic, research, and other purposes. Such uses include, for example, the following.

Constructs, including antigen-binding constructs of the invention may be used for immunohistochemistry applications. For example, they may be used for immunolocalization of a particular antigen or group of antigens in a tissue. Tissue can be fixed and incubated with antigen-binding constructs of interest. These constructs can then be localized using a secondary antibody or binding construct of the invention coupled to a label, for example, to a gold particle or an enzyme that gives a chemical reaction, like horseradish peroxidase or beta-galactosidase. A secondary antibody or binding construct is frequently made that is reactive against, for example, a portion of the primary binding construct. Thus, for example, if the primary binding construct has a human tail portion, the secondary antibody or binding construct could be, for example, a rabbit anti-mouse antibody or antigen-binding construct that has been linked to beta-galactosidase. Alternatively the antibody or binding construct of the invention can be purified and then conjugated to another molecule to produce a fluorescent antibody or binding construct.

Constructs, including antigen-binding constructs of the invention can also be used to detect the location of an antigen or antigens on the surface of cells or to detect the location of intracellular materials using, for example, Immunoelectron Microscopy. Electron dense materials such as ferritin or colloidal gold, for example, can be conjugated to an antigen-binding construct. Scanning electron microscopy can be used to detect the localization of the antigen/binding construct complex.

Constructs, including antigen-binding constructs of the invention may also be used to quantitate the presence of an antigen or antigens using one of a variety of immunoassay formats, for example, a radioimmunoassay (RIA) format or an enzyme-linked immunosorbent assay (ELISA) format. There are many variants of these approaches, but those are based on a similar idea. For example, if an antigen can be bound to a solid support or surface, or is in solution, it can be detected by reacting it with a specific antigen-binding construct of the invention. The presence or amount of the construct can then be detected or quantitated by reacting it with, for example, either a secondary antibody or a second antigen-binding construct of the invention by incorporating a label directly into the primary antibody. Alternatively, for example, an antigen-binding polypeptide of the invention can be bound to a solid surface and the antigen added. A second antibody or antigen-binding polypeptide(s) of the invention that recognizes a distinct epitope on the antigen can then be added and detected. This technique is commonly referred to as a "sandwich assay", which is frequently used to avoid problems of high background or non-specific reactions, among other reasons.

Because the binding constructs of the invention can have high affinity/affinities and/or selectivity/selectivities for a particular epitope or epitopes, they can also be used as affinity reagents, for example, in protein or antigen purification. In one example of such a process, antigen-binding constructs of the invention are immobilized on a suitable support, for example, Sephadex resin or filter paper. The immobilized construct is exposed to a sample containing, or suspected of containing, a target protein(s) or antigen(s). The support is rinsed with a suitable buffer that will remove unwanted materials. The support is washed with another buffer that will release the bound protein(s) or antigen(s).

Because particular binding constructs of the invention can bind to proteins or other antigens with high affinity and selectivity they can also be used as a criterion for the importance of a particular enzyme or other macromolecule in a particular reaction. If an antigen-binding construct of the invention can interfere with a reaction in a solution, this
5 will indicate that the construct may be binding specifically to a protein or other antigenic material involved in that reaction.

Constructs, including antigen-binding constructs of the invention can also be used as receptor blockers or inhibitors or antagonists.

Constructs, including antigen-binding constructs of the invention can also be
10 used in identifying and studying the function(s) of proteins. If an antigen-binding construct of the invention reacts with a specific protein, for example, that protein can subsequently be precipitated from solution, for example. Precipitation is typically performed by using a secondary antibody or antigen-binding construct of the invention that links primary complexes together. Alternatively, the complex can be removed by reacting the solution
15 with either protein A or, for example, depending on the construct, an anti-Fc antibody, for example, which has been attached to beads, for example, so that can be easily removed from the solution.

Constructs, including antigen-binding constructs of the invention can also be used in conjunction with gel-shift experiments to identify specific nucleic acid-binding
20 proteins such as DNA-binding proteins. For example, DNA-binding proteins can be assayed by their ability to bind with high affinity to a particular oligonucleotide. The mobility of an oligonucleotide associated with the protein is far different than the mobility of a free oligonucleotide and results in a gel migration pattern and signal that is commonly referred to as a gel shift. The addition of the construct to the binding assay can have either of two
25 effects. If the construct binds to a region of a protein not involved in DNA binding it can result in a complex that has even a slower mobility and is detected as a greater shift in mobility (a super-shift). Alternatively, if the construct binds to a region of the protein involved in recognizing the DNA then it can disrupt the binding and eliminate the shift. In either case, the data from these experiments can serve as a criterion to identify a DNA-
30 binding protein, for example.

It is also possible to use constructs, including antigen-binding constructs of the invention to detect a protein by western blotting after fractionation by SDS-PAGE, for example. Once fractionated proteins are transferred to a membrane such as a nitrocellulose sheet, they are exposed to a particular antigen-binding construct of the invention that specifically recognizes, or recognizes to a desired degree of selectivity, proteins immobilized to the blot. This allows particular proteins to be identified. This approach is particularly useful if the mobility of the protein changes during an experiment. For example, incorporation of a phosphate or a carbohydrate, or cleavage of the protein, results in a change in mobility that can be followed in straight forward manner by western analysis. With appropriate controls, this approach can be used to measure the abundance of a protein in response to experimental manipulations.

The combination of SDS gels and immunoprecipitation can also be extremely effective. If a particular protein can be immunoprecipitated in a solution, both supernatant and precipitated fractions can be separated on an SDS gel and studied using an antigen-binding constructs of the invention.

Sometimes a binding construct of the invention directed against one protein will also precipitate a second protein that interacts with the first protein. The second protein, as well as the first, can then be seen by staining the gel or by autoradiography. This relationship is frequently the first indication that a protein functions as part of a complex and it can also be used to demonstrate a physical interaction of two proteins that are hypothesized to interact on the basis of other evidence (*e.g.*, a two hybrid screen or a supressor mutation). This approach can be combined with western blotting analysis in several extremely effective ways.

Thus, for example, antigen-binding constructs of the invention can be used in a combination of immunoprecipitation and western analysis in the study, for example, of signal transduction and protein processing. For example, an immunoprecipitated protein can be subsequently studied by western analysis using a different antibody or antigen-binding construct of the invention that binds to the protein. The most useful of are those that are directed against particular structural determinants that may be present in a protein. Thus, an antibody or antigen-binding construct of the invention directed against a region of the

protein that undergoes proteolytic processing can be useful to follow proteolytic processing. Additionally, a construct of the invention or a mixture of antigen-binding constructs of the invention that recognize phosphorylated peptides (*e.g.*, anti PY (phosphorylated tyrosine) can be used to follow the extent of phosphorylation of a protein (using western analysis) after it is precipitated, or *visa versa*. Glycosylation reactions can also be followed by antigen-binding constructs of the invention directed against a carbohydrate epitope (or by lectins, *i.e.*, proteins that recognize carbohydrates). Likewise, some antigen-binding constructs of the invention can be made that specifically recognize a phosphorylated epitope, for example, that will recognize a tyrosine or a serine residue after phosphorylation, but will not bind (or detectably bind) the epitope in the absence of phosphate. This approach can be used to determine the phosphorylation state of a particular protein. For example, the phosphorylation of CREB (the cAMP response element binding protein) can be followed by an antibody that specifically recognizes an epitope in a way that is dependent on the phosphorylation of serine 133.

Constructs, including antigen-binding constructs of the invention can also be used to screen expression libraries to isolate candidate polynucleotides that express or present a particular epitope, or that have a particular affinity or expression characteristic.

Constructs, including antigen-binding constructs of the invention that bind to a cell surface can also be used as a marker to quantitate the fraction of cells expressing that marker using flow cytometry. If different antigen-binding constructs of the invention / fluorescent dye combinations are used, for example, the fraction of cells expressing several antigens can be determined.

Constructs, including antigen-binding constructs of the invention that function like anti-idiotypic antibodies, *i.e.*, antibodies against the binding domain of another antibody, can be used in any of a number of methods in which it would be desirable or useful to mimic the structure of an antigen. Such uses include, for example, uses as cancer vaccines (including antigen-binding constructs of the invention that incorporate a molecular adjuvant), as probes for receptors, as receptor agonists, as receptor antagonists, as receptor blockers or inhibitors, and so on.

In another aspect, constructs, including antigen-binding constructs of the invention may be bispecific and thus capable of binding to two distinct epitopes, which may be present on the same or different cell types.

In vivo uses of constructs of the invention, including antigen-binding constructs, include therapy, alone or in combination with one or more other therapies, for various diseases including cancers as well as B-cell disorders including autoimmune diseases. In some cases the constructs of the invention are administered to a patient. In other cases, the construct may be coupled to another molecule by techniques known in the art, for example, a fluorescent molecule to aid in imaging a target, or a therapeutic drug and/or a toxin to aid in killing a target.

For example, a labeling molecule or atom can be conjugated or otherwise linked to the antigen-binding construct of the invention to aid in imaging or as a diagnostic agent. These include, but are not limited to enzymatic labels, radioisotopes or radioactive compounds or elements, fluorescent compounds or metals, chemiluminescent compounds and bioluminescent compounds. Thus, binding constructs or antigen-binding constructs of the invention can be conjugated to a drug, which allows specific drug targeting and increased efficiency once the drug reaches the target. This facilitates drug therapy while reducing systemic toxicity and side effects. This allows use of drugs that would otherwise be unacceptable when administered systemically. Dosage will depend on the potency of the drug and the efficiency of the carrier construct. Other examples of *in vivo* uses include the use of binding constructs or antigen-binding constructs of the invention in which a toxin is chemically linked or conjugated to a polypeptide of the invention to form, for example, molecules that may be termed "immunoconjugates" or "immunotoxins." Typically, for example, such a toxin may include one or more radioisotopes (for example, Iodine-131, Yttrium-90, Rhenium-186, Copper-67, and/or Bismuth-212), natural toxins, chemotherapy agents, biological response modifiers, or any other substance that is capable of assisting in damaging or killing a target cell, inhibiting target cell replication, or is effective in disrupting a desired cellular function in a target cell.

The toxin portion of the immunotoxin can be derived from various sources. Toxins are commonly derived from plants or bacteria, but toxins of human origin or

synthetic toxins can be used as well, for example. Examples of toxins derived from bacteria or plants include, but are not limited to, abrin, α -sarcin, diphtheria toxin, ricin, saporin, and *pseudomonas* exotoxin. Examples of mammalian enzymes include, but are not limited to, ribonucleases (RNase) and deoxyribonucleases. Numerous immunotoxins that may be used
5 with one or more constructs of the invention have been described in the art. See, for example, U.S. Pat. No. 4,753,894 to Frankel *et al.*; U.S. Pat. No. 6,099,842 to Pastan *et al.*; Nevelle, *et al.*, 1982 *Immunol Rev.* **62**:75-91; Pastan *et al.*, 1992 *Ann Rev Biochem* **61**:331-354; Chaudary *et al.*, 1989 *Nature* **339**:394; and Batra *et al.*, 1991 *Mol. Cell. Biol.* **11**:2200. Modified toxins described herein and those described in the various publications are also
10 within the scope of the instant invention.

Generally, the immunotoxins and other therapeutic agents of this invention are administered at a concentration that is therapeutically effective to treat or prevent a particular disease, disorder, or condition, such as for the treatment of tumors and malignancies, the treatment of autoimmune diseases, allergies and inflammation, *etc.* This
15 effective dosage and mode of administration will depend on the animal or patient being treated, the disease or condition being treated, the strength of the immunoconjugates or immunotoxins and the efficiency of the conjugate. To accomplish this goal, the immunotoxins may be formulated using a variety of acceptable formulations and excipients known in the art. Typically, for example, the immunotoxins are administered by injection,
20 either intravenously or intraperitoneally. Methods to accomplish this administration are known to those of ordinary skill in the art. In another aspect, the invention includes topically or orally administered compositions such as an aerosol or cream or patch that may be capable of transmission across mucous membranes.

Formulants may be added to an immunoconjugates or immunotoxins of the
25 invention before administration to a patients being treated. A liquid formulation is most common, but other formulations are within the scope of the invention. The formulants may include for example oils, polymers, vitamins, carbohydrates, amino acids, salts, buffers, albumin, surfactants, or bulking agents. Carbohydrates can include sugar or sugar alcohols such as mono, di, or polysaccharides, or water-soluble glucans. The saccharides or glucans
30 can include for example fructose, dextrose, lactose, glucose, mannose, sorbose, xylose,

maltose, sucrose, dextran, pullulan, dextrin, alpha and beta cyclodextrin, soluble starch, hydroxethyl starch and carboxymethylcellulose, or mixtures thereof. "Sugar alcohol" may be defined as a C₄ to C₈ hydrocarbon having an -OH group and includes, for example, galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol. These sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used as long as the sugar or sugar alcohol is soluble in the aqueous preparation. In one aspect, the sugar or sugar alcohol concentration is between 0.5 w/v % and 15 w/v %, typically between 1.0 w/v % and 7.0 w/v %, more typically between 2.0 and 6.0 w/v %.

Exemplary amino acids include levorotary (L) forms of camitine, arginine, and betaine; however, other amino acids may be added. Commonly used polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, for example, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000, for example. A buffer can be used in the composition to minimize pH changes in the solution before lyophilization or after reconstitution. Any physiological buffer may be used, but citrate, phosphate, succinate, and glutamate buffers or mixtures thereof are more commonly utilized. The concentration can be, for example, from 0.01 to 0.3 molar. Higher or lower concentrations may be used.

Immunotoxins of the invention can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Exemplary polymers and methods to attach them to peptides are referenced in U.S. Pat. Nos. 4,766,106 to Katre *et al.*; 4,179,337 to Davis *et al.*; 4,495,285 to Shimizu *et al.*; and 4,609,546 to Hiratani.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1

CLONING OF THE 2H7 VARIABLE REGIONS AND CONSTRUCTION AND SEQUENCING OF 2H7scFv-IG

This Example illustrates the cloning of cDNA molecules that encode the heavy chain and light chain variable regions of the monoclonal antibody 2H7. This Example also demonstrates the construction, sequencing, and expression of 2H7scFv-Ig.

Hybridoma cells expressing 2H7 monoclonal antibody that specifically
5 bound to CD20 were provided by Ed Clark at the University of Washington, Seattle, WA. Prior to harvesting, hybridoma cells were kept in log phase growth for several days in RPMI 1640 media (Invitrogen/Life Technologies, Gaithersburg, MD) supplemented with glutamine, pyruvate, DMEM non-essential amino acids, and penicillin-streptomycin. Cells were pelleted by centrifugation from the culture medium, and 2×10^7 cells were used to prepare
10 RNA. RNA was isolated from the 2H7-producing hybridoma cells using the Pharmingen (San Diego, CA) total RNA isolation kit (Catalog # 45520K) according to the manufacturer's instructions accompanying the kit. One microgram (1 μ g) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA and 300 ng random primers were combined and denatured at 72 °C for 10 minutes prior to addition of enzyme.
15 Superscript II reverse transcriptase (Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 μ l in the presence of 5X second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 42°C for one hour.

The 2H7 cDNA generated in the randomly primed reverse transcriptase
20 reaction and V region specific primers were used to amplify by PCR the variable regions for the light and heavy chain of the 2H7 antibody. The V region specific primers were designed using the published sequence (Genbank accession numbers M17954 for V_L and M17953 for V_H) as a guide. The two variable chains were designed with compatible end sequences so that an scFv could be assembled by ligation of the two V regions after amplification and
25 restriction enzyme digestion.

A (gly₄ser)₃ peptide linker to be inserted between the two V regions was incorporated by adding the extra nucleotides to the antisense primer for the V_L of 2H7. A Sac I restriction site was also introduced at the junction between the two V regions. The sense primer used to amplify the 2H7 V_L, that included a HindIII restriction site and the light
30 chain leader peptide was 5'-gtc aag ctt gcc gcc atg gat ttt caa gtg cag att ttt cag c-3' (SEQ ID

NO:__). The antisense primer was 5'-gtc gtc *gag ctc* cca cct cct cca gat cca cca ccg ccc gag cca ccg cca cct ttc agc tcc agc ttg gtc *cc*-3' (SEQ ID NO:__). The reading frame of the V region is indicated as a bold, underlined codon. The Hind III and SacI sites are indicated by underlined italicized sequences.

5 The V_H domain was amplified without a leader peptide, but included a 5' SacI restriction site for fusion to the V_L and a BclI restriction site at the 3' end for fusion to various tails, including the human IgG1 Fc domain and the truncated forms of CD40 ligand, CD154. The sense primer was 5'-gct gct *gag ctc tca* ggc tta tct aca gca agt ctg g-3' (SEQ ID NO:__). The SacI site is indicated in italicized and underlined font, and the reading frame of the codon for the first amino acid of the V_H domain is indicated in bold, underlined type.
10 The antisense primer was 5'-gtt gtc *tga tca gag* acg gtg acc gtg gtc cc-3' (SEQ ID NO:__). The BclI site is indicated in italicized, underlined type, and the last serine of the V_H domain sequence is indicated in bold, underlined type.

 The scFv-Ig was assembled by inserting the 2H7 scFv HindIII-BclI fragment
15 into pUC19 containing the human IgG1 hinge, CH2, and CH3 regions, which was digested with restriction enzymes, HindIII and BclI. After ligation, the ligation products were transformed into DH5α bacteria. Positive clones were screened for the properly inserted fragments using the SacI site at the V_L-V_H junction of 2H7 as a diagnostic site. The 2H7scFv-Ig cDNA was subjected to cycle sequencing on a PE 9700 thermocycler using a
20 25-cycle program by denaturing at 96 °C for 10 seconds, annealing at 50 °C for 30 seconds, and extending at 72°C for 4 minutes. The sequencing primers were pUC forward and reverse primers and an internal primer that annealed to the CH2 domain human in the IgG constant region portion. Sequencing reactions were performed using the Big Dye Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, CA) according to
25 the manufacturer's instructions. Samples were subsequently purified using Centriscap columns (Catalog # CS-901, Princeton Separations, Adelphia, N.J.), the eluates dried in a Savant vacuum dryer, denatured in Template Suppression Reagent (PE-ABI), and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems). The sequence was edited, translated, and analyzed using Vector Nti version 6.0 (Informax, North Bethesda, MD) .
30 Figure 1 shows the cDNA and predicted amino acid sequence of the 2H7scFv-Ig construct.

EXAMPLE 2

EXPRESSION OF 2H7 SCFV-IG IN STABLE CHO CELL LINES

This Example illustrates expression of 2H7scFv-Ig in a eukaryotic cell line and characterization of the expressed 2H7scFv-Ig by SDS-PAGE and by functional assays, including ADCC and complement fixation.

The 2H7scFv-Ig HindIII-XbaI (~1.6 kb) fragment with correct sequence was inserted into the mammalian expression vector pD18, and DNA from positive clones was amplified using QIAGEN plasmid preparation kits (QIAGEN, Valencia, CA). The recombinant plasmid DNA (100 µg) was then linearized in a nonessential region by digestion with AscI, purified by phenol extraction, and resuspended in tissue culture media, Excell 302 (Catalog # 14312-79P, JRH Biosciences, Lenexa, KS). Cells for transfection, CHO DG44 cells, were kept in logarithmic growth, and 10⁷ cells harvested for each transfection reaction. Linearized DNA was added to the CHO cells in a total volume of 0.8 ml for electroporation.

Stable production of the 2H7 scFv-Ig fusion protein (SEQ. ID NO:10) was achieved by electroporation of a selectable, amplifiable plasmid, pD18, containing the 2H7 scFv-Ig cDNA under the control of the CMV promoter, into Chinese Hamster Ovary (CHO) cells (all cell lines from American Type Culture Collection, Manassas, VA, unless otherwise noted). The 2H7 expression cassette was subcloned downstream of the CMV promoter into the vector multiple cloning site as a ~1.6 kb HindIII-XbaI fragment. The pD18 vector is a modified version of pcDNA3 encoding the DHFR selectable marker with an attenuated promoter to increase selection pressure for the plasmid. Plasmid DNA was prepared using Qiagen maxiprep kits, and purified plasmid was linearized at a unique AscI site prior to phenol extraction and ethanol precipitation. Salmon sperm DNA (Sigma-Aldrich, St. Louis, MO) was added as carrier DNA, and 100 µg each of plasmid and carrier DNA was used to transfect 10⁷ CHO DG44 cells by electroporation. Cells were grown to logarithmic phase in Excell 302 media (JRH Biosciences) containing glutamine (4mM), pyruvate, recombinant insulin, penicillin-streptomycin, and 2X DMEM nonessential amino acids (all from Life Technologies, Gaithersburg, Maryland), hereafter referred to as "Excell 302 complete" media. Media for untransfected cells also contained HT (diluted from a 100X solution of

hypoxanthine and thymidine) (Invitrogen/Life Technologies). Media for transfections under selection contained varying levels of methotrexate (Sigma-Aldrich) as selective agent, ranging from 50 nM to 5 μ M. Electroporations were performed at 275 volts, 950 μ F. Transfected cells were allowed to recover overnight in non-selective media prior to selective
5 plating in 96 well flat bottom plates (Costar) at varying serial dilutions ranging from 125 cells/well to 2000 cells/well. Culture media for cell cloning was Excell 302 complete, containing 100 nM methotrexate. Once clonal outgrowth was sufficient, serial dilutions of culture supernatants from master wells were screened for binding to CD20-CHO transfected cells. The clones with the highest production of the fusion protein were expanded into T25
10 and then T75 flasks to provide adequate numbers of cells for freezing and for scaling up production of the 2H7scFvIg. Production levels were further increased in cultures from three clones by progressive amplification in methotrexate containing culture media. At each successive passage of cells, the Excell 302 complete media contained an increased concentration of methotrexate, such that only the cells that amplified the DHFR plasmid
15 could survive.

Supernatants were collected from CHO cells expressing the 2H7scFv-Ig, filtered through 0.2 μ m PES express filters (Nalgene, Rochester, NY) and were passed over a Protein A-agarose (IPA 300 crosslinked agarose) column (Repligen, Needham, MA). The column was washed with PBS, and then bound protein was eluted using 0.1 M citrate buffer,
20 pH 3.0. Fractions were collected and eluted protein was neutralized using 1M Tris, pH 8.0, prior to dialysis overnight in PBS. Concentration of the purified 2H7scFv-Ig (SEQ ID NO: __) was determined by absorption at 280 nm. An extinction coefficient of 1.77 was determined using the protein analysis tools in the Vector Nti Version 6.0 Software package (Informax, North Bethesda, MD). This program uses the amino acid composition data to
25 calculate extinction coefficients.

Production levels of 2H7scFv-Ig by transfected, stable CHO cells were analyzed by flow cytometry. Purified 2H7scFv-Ig to CHO cells was allowed to bind to CHO cells that expressed CD20 (CD20 CHO) and analyzed by flow cytometry using a fluorescein-conjugated anti-human IgG second step reagent (Catalog Numbers H10101 and
30 H10501, CalTag, Burlingame, CA). Figure 2 (top) shows a standard curve generated by

titration of 2H7scFv-Ig binding to CD20 CHO. At each concentration of 2H7scFv-Ig, the mean brightness of the fluorescein signal in linear units is shown. Supernatants collected from T flasks containing stable CHO cell clones expressing 2H7scFv-Ig were then allowed to bind to CD20 CHO and the binding was analyzed by flow cytometry. The fluorescein signal generated by 2H7scFv-Ig contained in the supernatants was measured and the 2H7scFv-Ig concentration in the supernatants was calculated from the standard curve (Figure 2, bottom).

Purified 2H7scFv-Ig (SEQ ID NO: __) was analyzed by electrophoresis on SDS-Polyacrylamide gels. Samples of 2H7scFv-Ig, purified by independent Protein A Agarose column runs, were boiled in SDS sample buffer without reduction of disulfide bonds and applied to SDS 10% Tris-BIS gels (Catalog # NP0301, Novex, Carlsbad, CA). Twenty micrograms of each purified batch was loaded on the gels. The proteins were visualized after electrophoresis by Coomassie Blue staining (Pierce Gel Code Blue Stain Reagent, Catalog #24590, Pierce, Rockford, IL), and destaining in distilled water. Molecular weight markers were included on the same gel (Kaleidoscope Prestained Standards, Catalog # 161-0324, Bio-Rad, Hercules, CA). The results are presented in Figure 3. The numbers above the lanes designate independent purification batches. The molecular weights in kilodaltons of the size markers are indicated on the left side of the figure. Further experiments with alternative sample preparation conditions indicated that reduction of disulfide bonds by boiling the protein in SDS sample buffer containing DTT or 2-mercaptoethanol caused the 2H7scFv-Ig to aggregate.

Any number of other immunological parameters may be monitored using routine assays that are well known in the art. These may include, for example, antibody dependent cell-mediated cytotoxicity (ADCC) assays, secondary *in vitro* antibody responses, flow immunocytofluorimetric analysis of various peripheral blood or lymphoid mononuclear cell subpopulations using well established marker antigen systems, immunohistochemistry or other relevant assays. These and other assays may be found, for example, in Rose *et al.* (Eds.), *Manual of Clinical Laboratory Immunology*, 5th Ed., 1997 American Society of Microbiology, Washington, DC.

The ability of 2H7scFv-Ig to kill CD20 positive cells in the presence of complement was tested using B cell lines Ramos and Bjab. Rabbit complement (Pel-Freez, Rogers, AK) was used in the assay at a final dilution of 1/10. Purified 2H7scFv-Ig was incubated with B cells and complement for 45 minutes at 37 °C, followed by counting of live and dead cells by trypan blue exclusion. The results in Figure 4A show that in the presence of rabbit complement, 2H7scFv-Ig lysed B cells expressing CD20.

The ability of 2H7scFv-Ig to kill CD20 positive cells in the presence of peripheral blood mononuclear cells (PBMC) was tested by measuring the release of ⁵¹Cr from labeled Bjab cells in a 4-hour assay using a 100:1 ratio of PBMC to Bjab cells. The results shown in Figure 4B indicated that 2H7scFv-Ig can mediate antibody dependent cellular cytotoxicity (ADCC) because the release of ⁵¹Cr was higher in the presence of both PBMC and 2H7scFv-Ig than in the presence of either PBMC or 2H7scFv-Ig alone.

EXAMPLE 3

EFFECT OF SIMULTANEOUS LIGATION OF CD20 AND CD40 ON GROWTH OF NORMAL B CELLS, AND ON CD95 EXPRESSION, AND INDUCTION OF APOPTOSIS

This example illustrates the effect of cross-linking of CD20 and CD40 expressed on the cell surface on cell proliferation.

Dense resting B cells were isolated from human tonsil by a Percoll step gradient and T cells were removed by E-rosetting. Proliferation of resting, dense tonsillar B cells was measured by uptake of ³[H]-thymidine during the last 12 hours of a 4-day experiment. Proliferation was measured in quadruplicate cultures with means and standard deviations as shown. Murine anti-human CD20 monoclonal antibody 1F5 (anti-CD20) was used alone or was cross-linked with anti-murine κ monoclonal antibody 187.1 (anti-CD20XL). CD40 activation was accomplished using soluble human CD154 fused with murine CD8 (CD154) (Hollenbaugh *et al.*, *EMBO J.* 11: 4212-21 (1992)), and CD40 cross-linking was accomplished using anti-murine CD8 monoclonal antibody 53-6 (CD154XL). This procedure allowed simultaneous cross-linking of CD20 and CD40 on the cell surface. The results are presented in Figure 5.

The effect of CD20 and CD40 cross-linking on Ramos cells, a B lymphoma cell line, was examined. Ramos cells were analyzed for CD95 (Fas) expression and percent apoptosis eighteen hours after treatment (no goat anti-mouse IgG (GAM)) and/or cross-linking (+GAM) using murine monoclonal antibodies that specifically bind CD20 (1F5) and CD40 (G28-5). Control cells were treated with a non-binding isotype control (64.1) specific for CD3.

Treated Ramos cells were harvested, incubated with FITC-anti-CD95, and analyzed by flow cytometry to determine the relative expression level of Fas on the cell surface after CD20 or CD40 cross-linking. Data is plotted as mean fluorescence of cells after treatment with the stimuli indicated (Figure 6A).

Treated Ramos cells from the same experiment were harvested and binding of annexin V was measured to indicate the percentage apoptosis in the treated cultures. Apoptosis was measured by binding of Annexin V 18 hours after cross-linking of CD20 and CD40 using 1F5 and G28-5 followed by cross-linking with GAM. Binding of Annexin V was measured using a FITC-Annexin V kit (Catalog # PN-IM2376, Immunotech, Marseille, France,). Annexin V binding is known to be an early event in progression of cells into apoptosis. Apoptosis, or programmed cell death, is a process characterized by a cascade of catabolic reactions leading to cell death by suicide. In the early phase of apoptosis, before cells change morphology and hydrolyze DNA, the integrity of the cell membrane is maintained but cells lose the asymmetry of their membrane phospholipids, exposing negatively charged phospholipids, such as phosphatidylserine, at the cell surface. Annexin V, a calcium and phospholipid binding protein, binds preferentially and with high affinity to phosphatidylserine. Results demonstrating the effect of cross-linking both CD20 and CD40 on expression of the FAS receptor (CD95) are presented in Figure 6B. The effect of cross-linking of both CD20 and CD40 on Annexin V binding to cells is shown in Figure 6B.

EXAMPLE 4

CONSTRUCTION AND CHARACTERIZATION OF 2H7 SCFV-CD154 FUSION PROTEINS

To construct a molecule capable of binding to both CD20 and CD40, cDNA encoding the 2H7 scFv was fused with cDNA encoding CD154, the CD40 ligand. The 2H7

scFv cDNA encoded on the HindIII-BclI fragment was removed from the 2H7 scFvIg construct, and inserted into a pD18 vector along with a BamHI-XbaI cDNA fragment encoding the extracellular domain of human CD154. The extracellular domain is encoded at the carboxy terminus of CD154, similar to other type II membrane proteins.

5 The extracellular domain of human CD154 was PCR amplified using cDNA generated with random primers and RNA from human T lymphocytes activated with PHA (phytohemagglutinin). The primer sets included two different 5' or sense primers that created fusion junctions at two different positions within the extracellular domain of CD154. Two different fusion junctions were designed that resulted in a short or truncated form (form
10 S4) including amino acids 108 (Glu)-261 (Leu) + (Glu), and a long or complete form (form L2) including amino acids 48 (Arg) -261 (Leu) + (Glu), of the extracellular domain of CD154, both constructed as BamHI-XbaI fragments. The sense primer which fuses the two different truncated extracellular domains to the 2H7scFv includes a BamHI site for cloning. The sense primer for the S4 form of the CD154 cDNA is designated SEQUENCE ID NO:
15 11 or CD154BAM108 and encodes a 34 mer with the following sequence : 5'-gtt gtc gga tcc aga aaa cag ctt tga aat gca a-3' , while the antisense primer is designated SEQUENCE ID NO: 12 or CD154XBA and encodes a 44 mer with the following sequence: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'.

 The oligonucleotide primers used in amplifying the long form (L2) of the
20 CD154 extracellular domain encoding amino acids 48 (Arg)-261 (Leu) + (Glu), were as follows: The sense primer designated CD154 BAM48 (SEQUENCE ID NO:13) encoded a 35-mer with the following sequence: 5'-gtt gtc gga tcc aag aag gtt gga caa gat aga ag-3'. The antisense primer designated or CD154XBA (SEQUENCE ID NO:__) encoded the 44-mer: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'. Other PCR reaction conditions
25 were identical to those used for amplifying the 2H7 scFv (see Example 1). PCR fragments were purified by PCR quick kits (QIAGEN, San Diego, CA), eluted in 30 µl ddH₂O, and digested with BamHI and XbaI (Roche) restriction endonucleases in a 40 µl reaction volume at 37 °C for 3 hours. Fragments were gel purified, purified using QIAEX kits according to the manufacturer's instructions (QIAGEN), and ligated along with the 2H7
30 HindIII-BclI fragment into the pD18 expression vector digested with HindIII+XbaI.

Ligation reactions were transformed into DH5-alpha chemically competent bacteria and plated onto LB plates containing 100 µg/ml ampicillin. Transformants were grown overnight at 37 °C, and isolated colonies used to inoculate 3 ml liquid cultures in Luria Broth containing 100 µg/ml ampicillin. Clones were screened after mini-plasmid preparations (QIAGEN) for insertion of both the 2H7 scFv and the CD154 extracellular domain fragments.

The 2H7scFv-CD154 construct cDNAs were subjected to cycle sequencing on a PE 9700 thermocycler using a 25-cycle program that included denaturing at 96 °C, 10 seconds, annealing at 50 °C for 5 seconds, and extension at 60°C, for 4 minutes. The sequencing primers used were pD18 forward (SEQ ID NO:__: 5'-gtctatataagcagagctctggc-3') and pD18 reverse (SEQ ID NO:__: 5'-cgaggctgatcagcgagctctagca-3') primers. In addition, an internal primer was used that had homology to the human CD154 sequence (SEQ ID NO:__: 5'-ccgcaatttgaggattctgatcacc-3'). Sequencing reactions included primers at 3.2 pmol, approximately 200 ng DNA template, and 8 µl sequencing mix. Sequencing reactions were performed using the Big Dye Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Samples were subsequently purified using Centrisep columns (Princeton Separations, Adelphia, NJ). The eluates were dried in a Savant speed-vacuum dryer, denatured in 20 µl template Suppression Reagent (ABI) at 95°C for 2 minutes, and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems). The sequence was edited, translated, and analyzed using Vector Nti version 6.0 (Informax, North Bethesda, MD). The 2H7scFv-CD154 L2 cDNA sequence and predicted amino acid sequence is presented in Figure 7A, and 2H7scFv-CD154 S4 cDNA sequence and predicted amino acid sequence is presented in Figure 7B.

The binding activity of the 2H7 scFv-CD154 fusion proteins (SEQ. ID NO: __ and __) to CD20 and CD40 simultaneously was determined by flow cytometry. The assay used CHO cell targets that express CD20. After a 45-minute incubation of CD20 CHO cells with supernatants from cells transfected with the 2H7 scFv-CD154 expression plasmid, the CD20 CHO cells were washed twice and incubated with biotin-conjugated CD40-Ig fusion protein in PBS/2% FBS. After 45 min, cells were washed twice and

incubated with phycoerythrin (PE)-labeled streptavidin at 1:100 in PBS/2% FBS (Molecular Probes, Eugene OR). After an additional 30 min incubation, cells were washed 2X and were analyzed by flow cytometry. The results show that the 2H7 scFv-CD154 molecule was able to bind to CD20 on the cell surface and to capture biotin-conjugated CD40 from solution (Figure 8).

To determine the effect of the 2H7scFv-CD154 on growth and viability of B lymphoma and lymphoblastoid cell lines, cells were incubated with 2H7scFv-CD154 L2 (SEQ. ID NO: __) for 12 hours and then examined for binding of Annexin V. Binding of Annexin V was measured using a FITC-Annexin V kit (Immunotech, Marseille, France, Catalog # PN-IM2376). B cell lines were incubated in 1 ml cultures with dilutions of concentrated, dialyzed supernatants from cells expressing secreted forms of the 2H7scFv-CD154 fusion proteins. The results are presented in Figure 9.

The growth rate of the Ramos B lymphoma cell line in the presence of 2H7scFv-CD154 was examined by uptake of ³H-thymidine for the last 6 hours of a 24-hour culture. The effect of 2H7scFv-CD154 on cell proliferation is shown in Figure 10.

EXAMPLE 5

CONSTRUCTION AND CHARACTERIZATION OF CYTOXB ANTIBODY DERIVATIVES

CytoxB antibodies were derived from the 2H7 scFv-IgG polypeptide. The 2H7 scFv (see Example 1) was linked to the human IgG1 Fc domain via an altered hinge domain (see Figure 11). Cysteine residues in the hinge region were substituted with serine residues by site-directed mutagenesis and other methods known in the art. The mutant hinge was fused either to a wild-type Fc domain to create one construct, designated CytoxB-MHWTG1C, or was fused to a mutated Fc domain (CytoxB-MHMG1C) that had additional mutations introduced into the CH2 domain. Amino acid residues in CH2 that are implicated in effector function are illustrated in Figure 11. Mutations of one or more of these residues may reduce FcR binding and mediation of effector functions. In this example, the leucine residue 234 known in the art to be important to Fc receptor binding, was mutated in the 2H7 scFv fusion protein, CytoxB-[MG1H/MG1C]. In another construct, the human IgG1 hinge region was substituted with a portion of the human IgA hinge, which was fused to wild-type

human Fc domain (CytoxB-IgAHWTHG1C). (See Figure 11). This mutated hinge region allows expression of a mixture of monomeric and dimeric molecules that retain functional properties of the human IgG1 CH2 and CH3 domains. Synthetic, recombinant cDNA expression cassettes for these molecules were constructed and polypeptides were expressed in CHODG44 cells according to methods described in Example 2.

Purified fusion protein derivatives of CytoxB-scFvIg molecules were analyzed by SDS-PAGE according to the methods described in Example 2. Polyacrylamide gels were run under non-reducing and reducing conditions. Two different molecule weight marker sets, BioRad prestained markers, (BioRad, Hercules, CA) and Novex Multimark molecular weight markers were loaded onto each gel. The migration patterns of the different constructs and of Rituximab™ are presented in Figure 12.

The ability of the different derivatives of CytoxB-scFvIg molecules to mediate ADCC was measured using the Bjab B lymphoma cells as the target and freshly prepared human PBMCs as effector cells. (See Example 2). Effector to target ratios were varied as follows: 70:1, 35:1, and 18:1, with the number of Bjab cells per well remaining constant but the number of PBMCs were varied. Bjab cells were labeled for 2 hours with ⁵¹Cr and aliquoted at a cell density of 5 x 10⁴ cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or rituximab were added at a concentration of 10 µg/ml to the various dilutions of PBMCs. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 4 hours, and 100 µl of culture supernatant was harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released. The results are presented in Figure 13.

Complement dependent cytotoxicity (CDC) activity of the CytoxB derivatives was also measured. Reactions were performed essentially as described in Example 2. The results are presented in Figure 14 as percent of dead cells to total cells for each concentration of fusion protein.

EXAMPLE 6

IN VIVO STUDIES IN MACAQUES

Initial *in vivo* studies with CytoxB derivatives have been performed in nonhuman primates. Figure 15 shows data characterizing the serum half-life of CytoxB in monkeys. Measurements were performed on serum samples obtained from two different macaques (J99231 and K99334) after doses of 6 mg/kg were administered to each monkey
5 on the days indicated by arrows. For each sample, the level of 2H7scFvIg present was estimated by comparison to a standard curve generated by binding of purified CytoxB-(MHWTG1C)-Ig fusion protein to CD20 CHO cells (see Example 2). The data are tabulated in the bottom panel of the Figure 15.

The effect of CytoxB-(MHWTG1C)Ig fusion protein on levels of circulating
10 CD40+ cells in macaques was investigated. Complete blood counts were performed at each of the days indicated in Figure 16. In addition, FACS (fluorescence activated cell sorter) assays were performed on peripheral blood lymphocytes using a CD40-specific fluorescein conjugated antibody to detect B cells among the cell population. The percentage of positive cells was then used to calculate the number of B cells in the original samples. The data are
15 graphed as thousands of B cells per microliter of blood measured at the days indicated after injection (Figure 16).

EXAMPLE 7

CONSTRUCTION AND EXPRESSION OF AN ANTI-CD19 scFv-IG FUSION PROTEIN

An anti-CD19 scFv-Ig fusion protein was constructed, transfected into
20 eukaryotic cells, and expressed according to methods presented in Examples 1, 2, and 5 and standard in the art. The variable heavy chain regions and variable light chain regions were cloned from RNA isolated from hybridoma cells producing antibody HD37, which specifically binds to CD19. Expression levels of a HD37scFv-IgAHWTG1C and a HD37scFv-IgMHWTG1C were measured and compared to a standard curve generated using
25 purified HD37 scFvIg. The results are presented in Figure 17.

EXAMPLE 8

CONSTRUCTION AND EXPRESSION OF AN ANTI-L6 scFv-IG FUSION PROTEIN

An scFv-Ig fusion protein was constructed using variable regions derived from an anti-carcinoma monoclonal antibody, L6. The fusion protein was constructed,

transfected into eukaryotic cells, and expressed according to methods presented in Examples 1, 2, and 5 and standard in the art. Expression levels of L6 scFv-IgAH WCH2 CH3 and L6 scFv-(SSS-S)H WCH2 WCH3 were measured and compared to a standard curve generated using purified L6 scFvIg. The results are presented in Figure 18.

5

EXAMPLE 9

CHARACTERIZATION OF VARIOUS SCFV-IG FUSION PROTEINS

In addition to the scFv-Ig fusion protein already described, G28-1 (anti-CD37) scFv-Ig fusion proteins were prepared essentially as described in Examples 1 and 5. The variable regions of the heavy and light chains were cloned according to methods known
10 in the art. ADCC activity of 2H7-MHWTG1C, 2H7-IgAHWTG1C, G28-1- MHWTG1C, G28-1 IgAHWTG1C, HD37- MHWTG1C, and HD37- IgAHWTG1C was determined according to methods described above (see Example 2). Results are presented in Figure 19. ADCC activity of L6scFv-IgAHWTG1C and L6scFv-IgMHWTG1C was measured using the 2981 human lung carcinoma cell line. The results are presented in Figure 20. The
15 murine L6 monoclonal antibody is known not to exhibit ADCC activity.

The purified proteins were analyzed by SDS-PAGE under reducing and non-reducing conditions. Samples were prepared and gels run essentially as described in Examples 2 and 5. The results for the L6 and 2H7 scFv-Ig fusion proteins are presented in Figure 21 and the results for the G28-1 and HD37 scFv-Ig fusion proteins are presented in
20 Figure 22.

EXAMPLE 10

CONSTRUCTION AND EXPRESSION OF ANTI-CD20 SCFV-LLAMA IG FUSION PROTEINS

This Example illustrates the cloning of llama IgG1, IgG2, and IgG3 constant region domains and the construction of immunoglobulin fusion proteins with each of the
25 three constant regions and anti-CD20 scFv.

The constant regions of llama IgG1, IgG2, and IgG3 immunoglobulins were cloned and inserted into mammalian vector constructs containing an anti-CD20 single chain Fv, 2H7 scFv. Total RNA was isolated from peripheral blood mononuclear cells (PBMC) from llama blood (Triple J Farms, Bellingham, WA) by lysing the lymphocytes in TRIzol®

(Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. One microgram (1 µg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA and 200 ng random primers were combined and denatured at 72 °C for 10 minutes prior to addition of enzyme. Superscript II reverse transcriptase (Invitrogen Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 µl in the presence of 5X second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 42 °C for one hour. The cDNA was amplified by PCR using sequence specific primers. The 5' primers were designed according to published sequences for the V_{HH} and V_H domains of camelids. The 3' primer, which was used to amplify all three isotypes, was designed using mammalian CH3 domain sequences as a guide. The following specific primers were used. The Bcl and XbaI sites are indicated by underlined italicized sequences.

5' primer for llama IgG1 constant region

LLG1-5'bgl: 5'-gtt gtt gat caa gaa cca cat gga gga tgc acg tg-3'

(SEQ ID NO:___)

5' primer for llama IgG2 constant region

LLG2-5'bgl: 5'-gtt gtt gat caa gaa ccc aag aca cca aaa cc-3'

(SEQ ID NO:___)

5' primer for llama IgG3 constant region

LLG3-5'bgl: 5'-gtt gtt gat caa gcg cac cac agc gaa gac ccc-3'

(SEQ ID NO:___)

3' primer for llama IgG1, IgG2, and IgG3 constant regions

LLG123-3'X: 5'-gtt gtt tct aga tta cta ttt acc cga aga ctg ggt gat gga-3'

(SEQ ID NO:___)

PCR fragments of the expected size were cloned into TOPO® cloning vectors (Invitrogen Life Technologies) and then were sequenced. The sense sequencing primer, LLseqsense, had the sequence 5'-ctg aga tcg agt tca gct g-3' (SEQ ID NO:___), and the antisense primer, LLseqAS, had the sequence 5'-cct cct ttg gct ttg tct c-3' (SEQ ID NO:___). Sequencing was performed as described in Example 1. Figure 23 compares the amino acid sequence of the three isotype llama constant regions containing the hinge, CH2,

and CH3 domains with the amino acid sequence of human IgG1 hinge, CH2, and CH3 domains.

After verifying the sequence, the amplified PCR products were digested with restriction enzymes BclI and XbaI to create compatible restriction sites. The digested fragments were then gel-purified, and the DNA was eluted using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA). The 2H7scFv-Ig pD18 mammalian expression vector construct (see Example 2) was digested with BclI and XbaI to remove the human IgG hinge, CH2, and CH3 domains. The pD18 vector is a modified derivative of pCDNA3 that contains an attenuated DHFR gene, which serves as a selectable marker for mammalian expression (Hayden *et al.*, *Tissue Antigens* 48:242-54 (1996)). The purified llama IgG1, IgG2, and IgG3 constant region PCR products were ligated by T4 DNA ligase (Roche Molecular Biochemicals, Indianapolis, IN) into the double-digested 2H7 scFv-pD18 vector at room temperature overnight according to the manufacturer's instructions. After ligation, the ligation products were transformed into *E. coli* DH5 α bacteria (BD Biosciences, Palo Alto, CA) and plated according to standard molecular biology procedures and manufacturer's instructions. Isolated colonies were chosen to screen for transformants containing the correct inserts.

For expression of the encoded polypeptides, plasmid DNA from positive clones was transiently transfected into COS-7 cells using DEAE-dextran (Hayden *et al.*, *Ther Immunol.* 1:3-15 (1994)). COS-7 cells were seeded at approximately 3×10^6 cells per 150 mm plate and grown overnight until the cells were about 75% confluent. Cells were then washed once with serum-free DMEM (Invitrogen Life Technologies, Grand Island, NY). Transfection supernatant (10 ml) containing 400 μ g/ml DEAE-dextran, 0.1 mM chloroquine, and 5 μ g/ml of the DNA constructs were added to the cells, which were then incubated at 37 °C for 3-4 hrs. After incubation, cells were pulsed with 10 ml of 10% dimethyl sulfoxide (DMSO) in 1x PBS at room temperature for 2 minutes. Cells were then placed back into fully supplemented DMEM/10% FBS (1% L-glutamine, 1% penicillin/streptomycin, 1% sodium pyruvate, 1% MEM essential amino acids) (Invitrogen Life Technologies). After 24 hours, the media was replaced with serum-free fully

supplemented DMEM (Invitrogen Life Technologies), and the cells were maintained up to 21 days with media changes every 3-4 days.

Ig-fusion proteins were purified by passing COS cell culture supernatants through Protein A Agarose (Repligen, Cambridge, MA) columns. After application of the culture supernatant, the Protein A columns were then washed with 1x PBS (Invitrogen Life Technologies). Bound Ig-fusion proteins were eluted with 0.1 M citric acid (pH 2.8), and the collected fractions were immediately neutralized with Tris base (pH 10.85). The fractions containing protein were identified by measuring the optical density (A_{280}) and then were pooled, dialyzed against 1x PBS, (Invitrogen Life Technologies) and filtered through a 0.2 μ m filter.

The purified Ig-fusion proteins were analyzed by SDS-PAGE. Aliquots of 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, and Rituxan® (Rituximab, anti-CD20 antibody, Genentech, Inc. and IDEC Pharmaceuticals Corp.) (provided by Dr. Oliver W. Press, Fred Hutchison Cancer Research Center, Seattle, WA) (5 μ g protein) were combined with 25 μ l 2x NuPAGE® SDS Sample Buffer (Invitrogen Life Technologies) (non-reduced samples). Samples of each protein were also prepared in reducing sample buffer containing 5% 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO). Molecular weight markers (Invitrogen Life Technologies) were applied to the gels in non-reducing buffer only. The proteins were fractionated on NuPAGE® 10% Bis-Tris gels (Invitrogen Life Technologies). After electrophoresis (approximately 1 hour), the gels were washed three times, five minutes each, with Distilled Water (Invitrogen Life Technologies) and then stained in 50 ml Bio-Safe Coomassie Stain (BioRad, Hercules, CA) overnight at room temperature. After a wash in Distilled Water, the gels were photographed. The migration pattern of each Ig-fusion protein is presented in Figure 24.

The ability of the 2H7 scFv-llama Ig fusion proteins to bind to cells expressing CD20 was demonstrated by flow cytometry. Serial dilutions starting at 25 μ g/ml of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, and 2H7 scFv-llama IgG3 were prepared and incubated with CD20-transfected (CD20+) CHO cells (from the laboratory of Dr. S. Skov, Institute of Medical Microbiology and Immunology, Copenhagen Denmark in 1% FBS 1x PBS media (Invitrogen Life Technologies) for one hour on ice. After the

incubation, the cells were then centrifuged and washed with 1% FBS in 1x PBS. To detect bound 2H7 scFv-llama Ig, the cells were incubated for one hour on ice with fluorescein-conjugated goat anti-camelid IgG (heavy and light chain) (1:100) (Triple J Farms). The cells were then centrifuged and resuspended in 1% FBS-1x PBS and analyzed using a
5 Coulter Epics XL cell sorter (Beckman Coulter, Miami, FL). The data (percent of maximum brightness) are presented in Figure 25.

EXAMPLE 11

EFFECTOR FUNCTION OF ANTI-CD20 scFv-LLAMA Ig FUSION PROTEINS

10 This Example demonstrates the ability of anti-CD20 llama IgG1, IgG2, and IgG3 fusion proteins to mediate complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC).

The ability of the 2H7 scFv-llama IgG fusion proteins to kill CD20 positive cells in the presence of complement was tested using the BJAB human B cell line. Rabbit
15 complement was obtained from 3-4 week old rabbits (Pel-Freez, Brown Deer, WI). BJAB cells (2×10^6 cells/ml) were combined with rabbit complement (final dilution 1:10) and purified 2H7 Ig fusion proteins. 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, and 2H7 scFv-human IgG1 wild type hinge-CH2-CH3) (Example 1) were added at 1:3 serial dilutions beginning at a concentration of 30 μ g/ml. After one hour at
20 37 °C, cell viability was determined by counting live and dead cells by trypan blue exclusion (0.4%) (Invitrogen Life Technologies) using a hemacytometer (Bright-line, Horsham, PA). The percent killing was calculated by dividing the number of dead cells by the number of total cells (dead + live cells). The data presented in Figure 26 show that all Ig fusion proteins had CDC activity.

25 The ADCC activity of the 2H7 scFv-llama IgG fusion proteins was determined using BJAB cells as target cells and human or llama peripheral blood mononuclear cells (PBMC) as effector cells. BJAB cells were pre-incubated for approximately 2 hours with ^{51}Cr (100 μ Ci) (Amersham Biosciences, Piscataway, NJ) in fully supplemented IMDM (Invitrogen Life Technologies) containing 15% FBS. The cells
30 were mixed intermittently during the pre-incubation period. Fresh, resting human PBMC

were purified from whole blood using Lymphocyte Separation Media (LSM) (ICN Pharmaceuticals, New York, NY). PBMC were combined with labeled BJAB cells (5×10^4 cells per well of 96 well tissue culture plate) at ratios of 25:1, 50:1, and 100:1. To each combination was added 10 $\mu\text{g/ml}$ of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, Rituximab, or no anti-CD20 antibody. The mixtures were incubated for 6 hours at 37 °C. Supernatant from each reaction containing ^{51}Cr released from lysed cells was collected onto a LumaPlate-96 filter plate (Packard, Meriden, CT), which was dried overnight. The amount of ^{51}Cr was measured by a TopCount NXT plate reader (Packard). Figure 27 shows that the 2H7 scFv-llama IgG2 fusion protein was the most effective llama fusion protein in mediating ADCC. Each data point represents the average measurement of triplicate wells.

ADCC activity was affected by the source of effector cells. Llama PBMC were isolated from llama blood (Triple J Farms) using LSM. Llama effector cells were added at the same ratios to BJAB target cells as described for the ADCC assay using human effector cells. The cells were combined with 10 $\mu\text{g/ml}$ of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, Rituximab, or no anti-CD20 antibody. The results are presented in Figure 28.

EXAMPLE 12

CONSTRUCTION AND CHARACTERIZATION OF SCFV IG FUSION PROTEINS

EXPRESSED ON THE CELL SURFACE

This Example describes a retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors. The Example also demonstrates the effector function of these various scFv Ig fusion proteins expressed on the surface of target cells.

The heavy and light chain variable regions were cloned from murine monoclonal antibodies specific for various costimulatory receptors, and single chain Fv constructs were prepared essentially as described in Example 1. Antibodies included 2H7, anti-human CD20; 40.2.220, anti-human CD40; 2E12, anti-human CD28; 10A8, anti-human CD152 (anti-CTLA-4); and 500A2, anti-murine CD3. The heavy chain and light chain variable regions of each antibody were cloned according to standard methods for cloning

immunoglobulin genes and as described in Example 1. Single chain Fv constructs were prepared as described in Example 1 by inserting a nucleotide sequence encoding a (gly₄ser)₃ peptide linker between the VL region nucleotide sequence of 40.2.220, 2E12, 10A8, and 500A2, respectively (SEQ ID NOs: __, respectively) and the VH region nucleotide sequence of 40.2.220, 2E12, 10A8, and 500A2, respectively (SEQ ID NOs: __, respectively). The polypeptide sequence for VL of 40.2.220, 2E12, 10A8, and 500A2 are set forth in SEQ ID NOs: __, respectively, and the polypeptide sequence for VH of 40.2.220, 2E12, 10A8, and 500A2 are set forth in SEQ ID NOs: __, respectively. Each scFv polynucleotide (SEQ ID NOs: __ for 40.2.220, 2E12, 10A8, and 500A2, respectively) was then fused to human IgG1 mutant hinge (CCC→SSS) and mutant CH2 (proline to serine mutation at residue 238 (238 numbering according to EU nomenclature, Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94; residue 251 according to Kabat *et al.*) and wild type CH3 domains according to the methods described in Example 5 and 11. Each scFv mutant IgG1 fusion polynucleotide sequence was then fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO: __), such that when the fusion protein was expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein. cDNAs encoding the scFv-IgG-CD80 fusion proteins (SEQ ID NOs: __ for 40.2.220-, 2E12-, 10A8-, and 500A2-scFv-IgG-CD80, respectively) were inserted into the retroviral vector pLNCX (BD Biosciences Clontech, Palo Alto, CA) according to standard molecular biology procedures and vendor instructions. The scFv-Ig-CD80 cDNA was inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence. The retroviral constructs were transfected into Reh, an acute lymphocytic leukemia cell line (ATCC CRL-8286). Transfected cells were screened to select clones that were expressing scFv-Ig fusion proteins on the cell surface.

CDC and ADCC assays were performed with the transfected Reh cells to determine if expression of the scFv-Ig polypeptides on the cell surface augmented effector cell function. Reh cells expressing anti-human CD152 scFv-mutant IgG-CD80 (SEQ ID NO: __); Reh anti-human CD28 scFv- mutant IgG-CD80 (SEQ ID NO: __); Reh anti-human CD28 scFv- mutant IgG-CD80 (SEQ ID NO: __); Reh anti-human CD40 scFv- mutant IgG-CD80 (SEQ ID NO: __); Reh anti-human CD20 scFv- mutant IgG-CD80 (SEQ ID NO: __)

were combined with human PBMC (see Example 11) and rabbit complement (10 μ g/ml) for one hour at 37 °C. Untransfected Reh cells were included as a control. Viability of the cells was determined by trypan blue exclusion, and the percent of killed cells was calculated (see Example 11). Figure 29 shows the effectiveness of the scFv-IgG-CD80 fusion proteins
5 when expressed on the cell surface of tumor cells to mediate complement dependent cytotoxicity.

The same transfected Reh cells tested in the CDC assay plus Reh cells transfected with the polynucleotide construct that encodes anti-murine CD3-scFv-Ig-CD80 (SEQ ID NO:___) were analyzed for ADCC activity (see Example 11). Untransfected and
10 transfected Reh cells were pre-labeled with 51 Cr (100 μ Ci) (Amersham) for two hours at 37 °C. Human PBMC served as effector cells and were added to the Reh target cells (5 x 10⁴ cells per well of 96 well plate) at ratios of 5:1, 2.5:1, and 1.25:1. After five hours at 37 °C, culture supernatants were harvested and analyzed as described in Example 11. Percent specific killing was calculated according to the following equation: ((experiment
15 release minus spontaneous release)/(maximum release minus spontaneous release)) x 100. The data are presented in Figure 30. Each data point represents the average of quadruplicate samples.

Using the same procedures described above, the same results with other binding domains were obtained using the following monoclonal antibodies monoclonal
20 antibodies as sources of sFv: for CD20, 1F5 (Genbank AY 058907 and AY058906); for CD40, 2.36 and G28.5; for CD28, 9.3.

Cell surface expression of antibody binding domains is accomplished by fusing antibody scFvs to IgA hinge and constant regions and IgE hinge-acting region, *i.e.*, IgE CH2, and constant regions. Polynucleotides encoding an anti-4-1BB scFv, 5B9 (anti-
25 human 4-1BB) scFv, and 2e12 (anti-human CD40) fused to IgAH IgA T4 (four terminal CH3 residues deleted) fused to the CD80 transmembrane and cytoplasmic domains and IgE Fc regions are shown in SEQ ID NOs:____. The encoded polypeptides are shown in SEQ ID NOs:____.

EXAMPLE 13

CONSTRUCTION AND SEQUENCE OF HUMAN IG HINGE-CH2-CH3 MUTANTS AND 2H7

VARIABLE REGION MUTANTS

This Example describes construction of scFv fusion proteins containing mutant human IgG1 and IgA constant regions. This Example also describes construction of a 2H7 scFv mutant with a single point mutation in the variable heavy chain region. Mutations were introduced into variable and constant region domains according to methods described herein and known in the molecular biology arts. Figure 31 presents nomenclature for the Ig constant region constructs.

The human IgG1 hinge region of the 2H7 scFv human IgG1 hinge-CH2-CH3 fusion proteins was mutated to substitute cysteine residues that in a whole immunoglobulin are involved in forming disulfide bonds between two heavy chain molecules. One mutant, 2H7 scFv fused to a human IgG1 hinge region in which all three cysteine residues were mutated to serine residues (MTH (SSS)), was prepared as described in Example 5 (designated in Example 5 as CytoxB-MHWTG1C (includes wild type IgG1 CH2 and CH3 domains)) (now referred to as 2H7 scFv MTH (SSS) WTCH2CH3) and comprises the polynucleotide sequence SEQ ID NO:___ encoding the polypeptide as set forth in SEQ ID NO___. The polynucleotide sequence encoding this mutant (SEQ ID NO:___) was used as a template to create mutant hinge regions in which the first two cysteine residues were substituted with serine residues (IgG MTH (SSC)). An oligonucleotide was designed to substitute the third serine residue with a cysteine and had the following sequence: 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca gca cct g-3' (HuIgGMHncs3, SEQ ID NO:___). A second mutant was prepared in which the mutant hinge had serine residues substituting the first and third cysteine residues (IgG MTH (SCS)). The sequence of the oligonucleotide to create this mutant was as follows: 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg-3' (HuIgGMHncs2, SEQ ID NO:___). A third mutant was prepared with cysteine residues substituted at the second and third positions (IgG MTH (CSS)), also using the IgG MTH (SSS) mutant as template, and an oligonucleotide having the sequence, 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac-3' (HuIgGMHncs1, SEQ ID NO:___).

The oligonucleotides introducing the mutations into the hinge region were combined with template and a 3' oligonucleotide containing an XbaI site (underlined and italicized) (5'-gtt gtt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (SEQ ID NO:___)) to amplify the mutant hinge-wild type (WT)-CH2-CH3 sequences by PCR. The IgG MTH CSS and IgG MTH SCS mutant sequences were amplified for 25 cycles with a denaturation profile of 94 °C, annealing at 52 °C for 30 seconds, and extension at 72 °C for 30 seconds. The IgG MTH SSC mutant sequence was amplified under slightly different conditions: denaturation profile of 94 °C, annealing at 45 °C for 30 seconds, and extension at 72 °C for 45 seconds. The amplified polynucleotides were inserted into the TOPO® cloning vector (Invitrogen Life Technologies) and then were sequenced as described in Example 1 to confirm the presence of the mutation. pD18 vector containing 2H7 scFv was digested to remove the constant region sequences essentially as described in Example 10. The mutant hinge-wild type CH2-CH3 regions were inserted in frame into the digested vector DNA to obtain vectors comprising 2H7 scFv MTH (CSS) WTCH2CH3 encoding DNA (SEQ ID NO:___); 2H7 scFv MTH (SCS) WTCH2CH3 encoding DNA (SEQ ID NO:___); and 2H7 scFv MTH (SSC) WTCH2CH3 encoding DNA (SEQ ID NO:___).

A mutation of leucine to serine at position 11 in the first framework region of the heavy chain variable region (numbering according to Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)) was introduced into the 2H7 scFv MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO:___). The wild type leucine residue was substituted with serine by site-directed mutagenesis using the oligonucleotide Vhser11: 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (SEQ ID NO:___). The 3'-primer for PCR was huIgG1-3' having the sequence 5'-gtc tct aga cta tca ttt acc cgg aga cag-3' (SEQ ID NO:___) (XbaI site underlined and italicized). After PCR amplification, the fragments were inserted into the TOPO® cloning vector and sequenced to confirm the presence of the VH11 leucine to serine mutation. The 2H7 scFv-IgG MTH (SSS) WTCH2CH3 encoding DNA was shuttled into the PSL1180 cloning vector (Pharmacia Biotech, Inc., Piscataway, NJ). The construct PSL1180-2H7 scFv-IgG MTH (SSS) WTCH2CH3 was digested with Sac and XbaI to remove the wild type VH domain and the hinge and CH2 and CH3

domains. The PCR product comprising the VH11 mutant was digested with Sac and XbaI and then inserted into the digested PSL1180 construct according to standard molecular biology procedures. The construct was then digested with Hind III and XbaI, and inserted into the mammalian expression vector pD18 (see methods described in Example 1 and
5 Example 10). The mutant is designated 2H7 scFv VH11SER IgG MTH (SSS) WTCH2CH3 (Figure 31). The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

Four constructs containing IgA constant region domains were prepared. One construct contained wild type IgA hinge fused to human IgG1 CH2 and CH3 (IgAH IgG
10 WTCH2CH3) (Figure 31). Sequential PCR amplifications were performed to substitute the human IgG1 hinge of the 2H7 scFv construct with nucleotide sequences encoding the IgA hinge. The 5' oligonucleotide primer (huIgA/Gchim5) for the first PCR reaction had the sequence, 5'-cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg-3' (SEQ ID NO:__). The primer (huIgAhg-5') for the second PCR reaction to add more IgA specific
15 hinge sequence and add a BclI restriction enzyme site (*italicized and underlined*) had the sequence, 5'-gtt *ggt gat cag* cca gtt ccc tca act cca cct acc cca tct ccc caa ct-3' (SEQ ID NO:__). The 3' primer for both amplification steps was huIgG1-3' having the sequence, 5'-gtc tct aga cta tca ttt acc cgg aga cag-3' (SEQ ID NO:__). The sequence of the PCR product was confirmed by TOPO ® cloning as described above. The gel-purified fragment
20 was digested with BclI and XbaI and then inserted into the 2H7 scFv-pD18 vector that had been digested BclI and XbaI to remove all the IgG1 constant region domains. Ligation was performed as described in Example 10 to provide a mammalian expression vector comprising the nucleotide sequence (SEQ ID NO:__) encoding a 2H7 scFv IgA hinge-IgG1 CH2-CH3 polypeptide (SEQ ID NO:__).

25 A second pD18 mammalian expression vector was constructed that had a polynucleotide sequence (SEQ ID NO:__) that encoded a 2H7 scFv fused to wild type IgA hinge, CH2, and CH3 domains (SEQ ID NO:__). Human IgA constant regions sequences were obtained by using random primers to reverse transcribe total RNA isolated from human tonsil followed by PCR amplification of the cDNA using sequence specific primers,
30 essentially as described in Example 10. Human IgA hinge-CH2-CH3 nucleotide sequence

(SEQ ID NO:____) encoding the IgA-CH2-CH3 polypeptide (IgAH IgACH2CH3, Figure 31) (SEQ ID NO:____) was amplified using the 5' oligonucleotide huIgAhg-5' (SEQ ID NO:(*same as above*____)) and a 3' oligonucleotide huIgA3' having the sequence, 5'-gtt gtt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac-3' (SEQ ID NO:____). Secretion of a

5 2H7-IgA hinge-IgA CH2-CH3 polypeptide from transfected mammalian cells required co-expression of human J chain that covalently binds to two IgA CH3 domains via disulfide bonds. Total RNA was isolated from tonsil B cells and was reversed transcribed to generate cDNA as described above. PCR amplification of the nucleotide sequence encoding the J chain was performed with J chain specific primers. The 5' PCR primer, HUIJCH5nl, had the

10 sequence, 5'-gtt gtt aga tct caa gaa gat gaa agg att gtt ctt-3' (SEQ ID NO:____), and sequence of the 3' primer, HUIJCH3, was 5'-gtt gtt tct aga tta gtc agg ata gca ggc atc tgg-3' (SEQ ID NO:____). The cDNA was cloned into TOPO® for sequencing as described in Example 10. J chain encoding cDNA (SEQ ID NO:____) was then inserted into pD18 and pCDNA3-Hygro (+) (Invitrogen Life Technology) vectors for co-transfection with 2H7 scFv IgA

15 hinge-CH2-CH3 constructs. The J chain has the predicted amino acid sequence set forth in SEQ ID NO:____.

Secretion of an scFv IgA constant region construct in the absence of J chain was accomplished by engineering a truncated CH3 domain with a deletion of the four carboxy terminal amino acids (GTCY, SEQ ID NO:____) (IgAH IgA-T4, Figure 31), which

20 include a cysteine residue that forms a disulfide bond with the J chain. The IgA hinge-CH2-CH3 nucleotide sequence containing the deletion in CH3 (SEQ ID NO:____) was prepared using a 5' PCR primer (huIgAhg-5') having the sequence 5'-gtt gtt gat cag cca gtt ccc tca act cca cct acc cca tct ccc tca act-3' (SEQ ID NO:____) (BclI site is underlined and italicized), and a 3' PCR primer (HUIGA3T1) having the sequence 5'-gtt gtt tct aga tta tca gtc cac ctc

25 cgc cat gac aac aga cac-3' (SEQ ID NO:____). This mutated IgA constant region nucleotide sequence was inserted into a 2H7 scFv pD18 vector as described for the generation of the previous 2H7 scFv-Ig constructs (see Example 1 and this example) that comprises the polynucleotide sequence (SEQ ID NO:____) encoding a 2H7 IgAH IgA-T4 polynucleotide (SEQ ID NO:____).

A fourth construct was prepared that encoded a 2H7 scFv-IgA constant region fusion protein with a deletion of 14 additional amino acids, most of which are hydrophobic residues, from the carboxy terminus of IgA CH3. The 2H7 scFv-IgAH IgA-T4 encoding polynucleotide was used as template to engineer a deletion of the nucleotide sequence encoding PTHVNVSVVMAEVD (SEQ ID NO:___). The 5' oligonucleotide primer had the sequence 5'-gtt ggt gat cag cca gtt ccc tca act cca cct acc cca tct ccc tca act-3' (SEQ ID NO:___) (BclI site shown as underlined and italicized). The 3' oligonucleotide sequence was 5'-gtt gtt tct aga tta tca ttg acc cgc caa gcg gtc gat ggt ctt-3' (SEQ ID NO:___). The deleted IgA CH3 region was amplified by using the above oligonucleotides to amplify the IgA constant region from RNA isolated from human tonsil such that the cDNA contained the deleted carboxyl terminal encoding region for the 18 amino acids. The IgAH IgA-T18 constant region was inserted into a 2H7 scFv pD18 vector that comprises the polynucleotide sequence (SEQ ID NO:___) encoding a 2H7 IgAH IgA-T18 polynucleotide (SEQ ID NO:___) as described above.

EXAMPLE 14

EFFECTOR FUNCTION OF CTLA-4 IGG FUSION PROTEINS

The Example compares the effector functions of CTLA-4 Ig fusion proteins in CDC and ADCC assays.

Two CTLA-4 IgG fusion proteins were constructed. One fusion protein comprises the extracellular domain of CTLA-4 fused to human IgG1 wild type hinge, CH2, and CH3 domains and is designated CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___). A pD18 mammalian expression vector comprising a polynucleotide sequence encoding CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___) was prepared by fusing in frame the nucleotide sequence encoding the extracellular domain of CTLA-4 (SEQ ID NO:___) (see U.S. Patent No. 5,844,095) to the nucleotide sequence encoding IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___) according to the methods described in Examples 1 and 10. The extracellular domain nucleotide sequence also comprises a BclI restriction enzyme site at the 3' end, and a leader peptide nucleotide sequence (SEQ ID NO:___) that encodes an oncoM leader peptide (SEQ ID NO:___). A second CTLA-4 IgG fusion protein,

designated CTLA-4 IgG MTH (SSS) MTCH2WTCH3, contained the extracellular domain of CTLA-4 (plus the oncoM leader peptide sequence) fused to a mutant IgG hinge in which all three cysteine residues were replaced with serine residues. The hinge region was fused to a mutant IgG1 CH2 domain that had a mutation at isotype position 238 (EU numbering, Ward *et al.*, *supra*, (position 251 using numbering according to Kabat *et al.*, *supra*; position 209 where numbering commences with first residue of IgG1 CH1; *i.e.*, PAPELLDGPS (SEQ ID NO:__) of wild type IgG1 CH2 is modified to PAPELLDGSS (SEQ ID NO:__)), which was fused to IgG1 wild type CH3 (U.S. Patent No. 5,844,095). The CTLA-4 IgG MTH (SSS) MTCH2WTCH3 polynucleotide comprises the nucleotide sequence in SEQ ID NO:__ and the deduced amino acid sequence comprises the sequence provided in SEQ ID NO:__. CTLA-4 fusion proteins were also prepared using CTLA-4 extracellular membrane encoding sequences without the leader peptide (SEQ ID NO:__).

To measure CDC activity, purified CTLA-4 IgG WTH (CCC) WTCH2CH3 (2 µg/ml) or CTLA-4 IgG MTH (SSS) MTCH2WTCH3 (2 µg/ml) was added to Reh cells (see Example 12) and to Reh cells transfected with the costimulatory molecule CD80 such that CD80 was expressed on the cell surface (Reh CD80.10, obtained from Dr. E. Clark, University of Washington, Seattle, WA; see Doty *et al.*, 1998 *J. Immunol.* 161:2700; Doty *et al.*, 1996 *J. Immunol.* 157:3270), in the presence or absence of rabbit complement (10 µg/ml). Purified CTLA Ig fusion proteins were prepared from culture supernatants of transiently transfected COS cells according to methods described in Example 10. The assays were performed essentially as described in Example 11 and 12. The data presented in Figure 32 show that only CD80-transfected Reh cells were killed in the presence of complement and CTLA-4 IgG WTH (CCC) WTCH2CH3 fusion protein.

The purified CTLA-4 Ig fusion proteins were also tested in ADCC assays. Human PBMC, serving as effector cells, were added to Reh or Reh CD80.1 target cells at a ratio of 1.25:1, 2.5:1, 5.0:1, and 10:1. Cells were labeled and the assays performed essentially as described in Examples 11 and 12. The results are presented in Figure 33. Each data point represents the average of four independent culture wells at each effector:target cell ratio. The data show that only CTLA-4 IgG WTH (CCC) WTCH2CH3 mediated significant ADCC of Reh CD80.10 cells.

EXAMPLE 15

EFFECTOR FUNCTION OF CTLA-4 IGA FUSION PROTEINS

CTLA-4 IgA fusion proteins are prepared as described for the IgG fusion proteins (see Examples 1, 13, and 14). CTLA-4 extracellular domain nucleotide sequence (SEQ ID NO:___) is fused in open reading frame to nucleotides encoding IgAH IgACH2CH3 (SEQ ID NO:___) to provide the nucleotide sequence (SEQ ID NO:___) encoding a CTLA-4 IgAH IgACH2CH3 fusion protein (SEQ ID NO:___). The fusion protein is transiently expressed in COS cells (see Example 10) or stably expressed in CHO cells (see Example 1). Secretion of the CTLA-4 IgAH IgACH2CH3 fusion protein requires co-transfection with a construct containing a polynucleotide sequence (SEQ ID NO:___) that encodes human J chain (SEQ ID NO:___). The CTLA-4 IgAH IgACH2CH3 fusion protein is isolated as described in Examples 10 and 14. To express a CTLA-4 IgA construct without the presence of J chain, a CTLA-4 IgAH IgA-T4 construct is prepared and transfected into mammalian cells. In a similar manner as described for the CTLA-4 extracellular fragment fused to wild type IgA hinge-CH2CH3, the CTLA-4 extracellular domain nucleotide sequence (SEQ ID NO:___) is fused in open reading frame to a nucleotide sequence (SEQ ID NO:___) encoding a IgAH IgA-T4 polypeptide (SEQ ID NO:___) to provide a nucleotide sequence comprising SEQ ID NO:___ encoding a CTLA-4 IgAH IgA-T4 polypeptide (SEQ ID NO:___). Effector function of each construct is evaluated by CDC and ADCC as described in Example 14.

EXAMPLE 16

BINDING OF ANTI-CD20 scFv HUMAN Ig FUSION PROTEINS

TO CHO CELLS EXPRESSING CD20

This Example describes binding of 2H7 scFv Ig fusion proteins to CHO cells that express CD20. The analysis was performed by flow cytometry. Culture supernatants were collected from transiently transfected COS cells expressing 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___); 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:___); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:___); and 2H7 scFv VHSE11 WTH WTCH2CH3 (SEQ ID NO:___), and two-fold serial dilutions were

prepared. Serial two-fold dilutions of purified 2H7 scFv IgG MTH (SSC) WTCH2CH3 (SEQ ID NO:___) were prepared starting at a concentration of 5 µg/ml. The culture supernatants and purified fusion protein samples were incubated with (CD20+) CHO cells for one hour on ice. The cells were washed twice and then incubated with 1:100 FITC-conjugated goat anti-human IgG (CalTag) for 40 minutes. The unbound conjugate was then removed by washing the cells and flow cytometry analysis was performed using a Coulter Epics XL cell sorter. Results are presented in Figure 34.

EXAMPLE 17

IMMUNOBLOT ANALYSIS OF ANTI-CD20 scFv HUMAN IgG AND IGA FUSION PROTEINS

10 This Example describes immunoblot analysis of 2H7 scFv IgG and 2H7 scFv IgA fusion proteins that were immunoprecipitated from transfected cell culture supernatants.

 COS cells were transiently transfected with plasmids comprising nucleotide sequences for 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgA H IgG WTCH2CH3 (SEQ ID NO:); and scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:) essentially according to the method described in Example 10. Cells were also transfected with vector only. After 48-72 hours at 37°C, cell culture supernatants were harvested and combined with protein A-agarose beads (Repligen) for one hour at 4 °C. The beads were centrifuged and washed several times in TNEN [20 mM Tris base, 100 mM NaCl, 1 mM EDTA, and 0.05% NP-40, pH 8.0). The immunoprecipitates were combined with 25 µl 2x NuPAGE® SDS Sample Buffer (Invitrogen Life Technologies) (non-reduced samples). The proteins were fractionated on NuPAGE® 10% Bis-Tris gels (Invitrogen Life Technologies). After electrophoresis (approximately 1 hour), the proteins were transferred from the gel onto a Immobilon P polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA) using a semi-dry blotter (Ellard Instrumentation, Monroe, WA). The PVDF membrane was blocked in PBS containing 5% nonfat milk and then probed with HRP-conjugated goat anti-human IgG (Fc specific) (CalTag). After washing the immunoblot several times in PBS, the blot was developed using ECL (Amersham Biosciences). The results are shown in Figure 35.

EXAMPLE 18

BINDING OF ANTI-CD20 scFv HUMAN IGA FUSION PROTEINS TO CD20+ CHO CELLS

This Example describes flow immunocytofluorimetry analysis of binding of 2H7 scFv IgAH IgACH2CH3 (SEQ ID NO:___) and 2H7 scFv IgAH IgAT4 (SEQ ID NO:___) fusion proteins to (CD20+) CHO cells.

COS cells were transiently co-transfected as described in Example 10 with plasmid DNA comprising a polynucleotide sequence (SEQ ID NO:___) encoding 2H7 scFv IgAH IgACH2CH3 polypeptide (SEQ ID NO:___) and with a separate plasmid comprising a polynucleotide sequence (SEQ ID NO:___) encoding a human J chain polypeptide (SEQ ID NO:___). COS cells were also transfected with a polynucleotide sequence (SEQ ID NO:___) encoding a 2H7 scFv IgA fusion protein that had a deletion of four amino acids at the carboxy terminus of CH3 (2H7 scFv IgAH IgA-T4, SEQ ID NO:___). The transfections were performed as described in Example 10. Culture supernatants from transfected COS cells were combined with (CD20+) CHO cells (see Example 1) and incubated for one hour on ice. The cells were washed twice with PBS-2%FBS and then combined with FITC-conjugated goat anti-human IgA chain (CalTag) (1:100) for 40 minutes. The cells were again washed and then analyzed by flow cytometry using a Coulter Epics XL cell sorter. Figure 36 shows that co-transfection with J chain was not required for secretion of 2H7 scFv IgAH IgAT4, the 2H7 IgA fusion protein with the truncated CH3 carboxy end (SEQ ID NO:___).

EXAMPLE 19

EFFECTOR FUNCTION OF ANTI-CD20 scFv HUMAN IGA FUSION PROTEINS

This Example illustrates ADCC activity of 2H7 IgG and IgA fusion proteins against cells that express CD20. BJAB cells were prelabeled with ⁵¹Cr (100 μCi) (Amersham) for two hours at 37 °C. Effector cells were obtained from fresh, resting human whole blood, which was diluted in an equal volume of Alsever's solution to prevent coagulation. 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:___); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:___); 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___); and 2H7 scFv IgAH IgACH2CH3 (SEQ ID NO:___) fusion proteins were

purified from transiently transfected COS cell supernatants (100-200 ml) by protein A chromatography as described in Example 10. COS cells transfected with the plasmid encoding 2H7 scFv IgAH IgACH2CH3 were co-transfected with a plasmid encoding human J chain as described in Example 18. Two-fold serial dilutions of the purified 2H7 Ig fusion proteins starting at 5 μ g/ml were added to the labeled BJAB cells (5×10^4 cells per well of 96 well tissue culture plate) in the presence of whole blood (100 μ l of whole blood diluted 1:1 in Alsever's solution, final dilution 1:4) and incubated for five hours at 37 °C. Culture supernatants were harvested and analyzed as described in Example 11. Percent specific killing was calculated according to the following equation: ((experiment release minus spontaneous release)/(maximum release minus spontaneous release)) x 100. The data are presented in Figure 37. Each data point represents the average of quadruplicate samples.

In a second ADCC assay, the number of labeled BJAB target cells was held constant in each sample, and whole blood was added at dilutions of 0.25, 0.125, and 0.0625. Purified 2H7 IgG and IgA fusion proteins were added at a concentration of 5 μ g/ml. The BJAB cells, whole blood, and fusion proteins were incubated, the supernatants harvested, and the percent specific killing was calculated as described above. Percent specific killing for each of the 2H7 fusion proteins is presented in Figure 38.

The ADCC activity of purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 (5 μ g/ml) and of purified 2H7 scFv IgAH IgACH2CH3 (5 μ g/ml) was compared in the presence of different effector cell populations. PBMC were isolated from whole blood as described in Examples 11 and 12. PBMC were combined with labeled BJAB target cells (5×10^4 per well of 96 well tissue culture plate) at ratios of 50:1, 25:1, and 12.5:1. The assay was performed and the data analyzed as described above. Figure 39A shows that only the 2H7 scFv IgG MTH (SSS) WTCH2CH3 fusion protein had ADCC activity when PBMC served as the effector cells. Figure 39B shows that both 2H7 scFv IgG MTH (SSS) WTCH2CH3 and 2H7 scFv IgAH IgACH2CH3 exhibit ADCC activity when whole blood was the source of effector cells (as illustrated in Figure 38).

EXAMPLE 20

EXPRESSION LEVEL OF 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 FUSION

PROTEIN

This Example compares the expression level of 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO:___) with other 2H7 scFv IgG constructs that do not contain the mutation in the variable heavy chain domain. The mammalian expression vector pD18 comprising nucleotide sequences 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:); and 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 (see Examples 1 and 13) were transiently transfected into COS cells as described in Example 10. After 72 hours at 37 °C, culture supernatants were harvested, and 1 µl of each supernatant was combined with non-reducing sample buffer (see method described in Example 10). The culture supernatant samples and aliquots of purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 (40 ng, 20 ng, 10 ng/ 5 ng, and 2.5 ng) were fractionated on 10% Bis-Tris (MOPS) NuPAGE® gels (Invitrogen Life Technologies). Multimark® protein standards (Invitrogen Life Technologies) were also separated on the gel. The proteins were transferred to a PDVF membrane and immunoblotted as described in Example 17. The immunoblot is presented in Figure 40. The amounts of the fusion proteins were quantified by densitometry analysis of the blots using the ScionImage for Windows software and comparison with the standard curve. The 2H7 scFv IgG WTH (CCC) WTCH2CH3 construct produced approximately 12 ng/ul or 12 micrograms/ml, the 2H7 scFv IgG MTH (CSS) WTCH2CH3 produced approximately 10 ng/ul or 10 micrograms/ml, the 2H7 scFv IgG MTH (SCS) WTCH2CH3 construct produced approximately 1 ng/ul or 1 microgram/ml, and the 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 construct produced approximately 30 ng/ml or 30 micrograms/ml.

EXAMPLE 21

CONSTRUCTION OF A 2H7 scFv IgG FUSION PROTEIN WITH A MUTANT CH3 DOMAIN

Amino acid mutations were introduced into the CH3 domain of a 2H7 IgG fusion protein. The pD18 vector comprising 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ

ID NO:__) was digested with BclI and XbaI to remove the MTH WTCH2CH3 (SEQ ID NO:__) fragment, which was then subcloned into pShuttle vector (BD Biosciences Clontech, Palo Alto, CA) that was double-digested with BclI and XbaI. Subcloning was performed in a kanamycin resistant vector because the ampicillin resistance gene has an XmnI site, which is required for this cloning procedure. Five constructs were prepared with the following substitutions: (1) a phenylalanine residue at position 405 (numbering according to Kabat *et al. supra*) was substituted with tyrosine using the oligonucleotide CH3Y405; (2) the phenylalanine position at 405 was substituted with an alanine residue using the oligonucleotide CH3A405; (3) the tyrosine residue at position 407 was substituted with an alanine using the oligonucleotide CH3A407; (4) both wild type amino acids at positions 405 and 407 were substituted with tyrosine and alanine, respectively using the oligonucleotide CH3Y405A407; and (5) both wild type amino acids at positions 405 and 407 were substituted with alanine using the oligonucleotide CH3A405A407. The oligonucleotides were the 3' primers for PCR amplification of a portion of the CH3 domain. The nucleotide sequences for each 3' oligonucleotide were as follows.

CH3Y405: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag gta gaa gga gcc-3' (SEQ ID NO:__)

CH3A405: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag ggc gaa gga gcc-3' (SEQ ID NO:__)

CH3A407: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gaa gaa gga gcc-3' (SEQ ID NO:__)

CH3Y405A407: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gta gaa gga gcc-3' (SEQ ID NO:__)

CH3A405A407: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag ggc gaa gga gcc-3' (SEQ ID NO:__)

The template was the mutant hinge MHWTCCH2CH3 human IgG1. The 5' PCR oligonucleotide primer was huIgGMHWC, [SEQ ID NO:__]. The amplified products were TOPO® cloned and sequenced as described in Examples 1 and 10. DNA from the

clones with the correct sequence was digested with BclI and XmnI and transferred to pShuttle containing the MTH WTCH2CH3 sequence, which was also digested with the same restriction enzymes. The mutated IgG sequences were then removed by digestion with BclI and XbaI and inserted into a pD18 vector containing 2H7 scFv that was also digested with BclI and XbaI. The polynucleotide sequences for mutated the CH3 domains, MTCH3 Y405, MTCH3 A405, MTCH3 A407, MTCH3 Y405A407, and MTCH3 A405A407 are shown in SEQ ID NOs: ____, respectively, and the polypeptide sequences for each are shown in SEQ ID NOs: ____, respectively. The polynucleotide sequences for the 2H7 scFv MTH WTCH2 MTCH3 Y405, 2H7 scFv MTH WTCH2 MTCH3 A405, scFv MTH WTCH2 MTCH3 A407, scFv MTH WTCH2 MTCH3 Y405A407, and scFv MTH WTCH2 MTCH3 A405A407, respectively, and the deduced amino acid sequences are shown in SEQ ID NOs: ____, respectively.

EXAMPLE 22

CONSTRUCTION OF 2H7 scFv IgG FUSION PROTEINS WITH HINGE MUTATIONS

A 2H7 scFv IgG fusion protein was constructed with the third cysteine residue in the IgG1 hinge region substituted with a serine residue. The template for introduction of the mutations was a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO: ____). The oligonucleotide introducing the mutations was a 5' PCR primer oligonucleotide HIgGMHcys3 having the sequence 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tcc cca gca cct-3'. The oligonucleotide introducing the mutation into the hinge region was combined with template and a 3' oligonucleotide containing an XbaI site (underlined and italicized) (5'-gtt gtt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (SEQ ID NO: ____)) to amplify the mutant hinge-wild type (WT)-CH2-CH3 sequences by PCR. The IgG MTH CCS mutant sequence was amplified for 30 cycles with a denaturation profile of 94 °C, annealing at 50 °C for 30 seconds, and extension at 72 °C for 30 seconds. The amplified polynucleotides were inserted into the TOPO® cloning vector (Invitrogen Life Technologies) and then were sequenced as described in Example 1 to confirm the presence of the mutation. pD18 vector containing 2H7 scFv was digested to remove the constant region sequences essentially as described in Example 10. The mutant

hinge-wild type CH2-CH3 regions were inserted in frame into the digested vector DNA to obtain vectors comprising 2H7 scFv MTH (CCS) WTCH2CH3 encoding DNA (SEQ ID NO:___). The deduced polypeptide sequence is shown in SEQ ID NO:___.

EXAMPLE 23

5 CONSTRUCTION OF ANTI-CD20 IGE FUSION PROTEINS

A binding domain is fused to IgE constant region sequences such that the expressed polypeptide is capable of inducing an allergic response mechanism. The single chain Fv nucleotide sequence of 40.2.220 (SEQ ID NO:___), an anti-CD40 antibody, is fused to IgE CH2-CH3-CH4 according to methods described for other scFv immunoglobulin
10 constant region constructs (see Examples 1, 5, 10, and 13). To PCR amplify the IgE CH2-CH3-CH4 domains, a 5' oligonucleotide primer, hIgE5Bcl, having the sequence 5'-gtt gtt gat cac gtc tgc tcc agg gac ttc acc cc-3', and a 3' oligonucleotide primer, hIgE3stop, having the sequence 5'- gtt gtt tct aga tta act ttt acc ggg att tac aga cac cgc tcg ctg g-3' are used.

The retroviral transfection system for ectopic surface expression of
15 genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors described in Example 12 is used to construct a 40.2.220 scFv IgE-CD80 fusion protein. The 40.2.220 scFv IgE fusion polynucleotide sequence is fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO:___), such that when the fusion protein is expressed in the transfected cell, CD80
20 provided an anchor for surface expression of the scFv Ig fusion protein. cDNA encoding the anti-CD40 scFv-IgE-CD80 fusion proteins (SEQ ID NO:___) is inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The 40.2.220 scFv-Ig-CD80 cDNA is inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence.
25 The retroviral constructs are transfected into a carcinoma cell line, and transfected cells are screened to select clones that are expressing the 40.2.220 scFv-Ig-CD80 fusion protein on the cell surface.

EXAMPLE 24

CONSTRUCTION OF IGA-T4 MUTANTS THAT ARE EXPRESSED ON THE CELL SURFACE

The retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors described in Example 12 is used to construct a 2H7 scFv IgA hinge IgA-T4-CD80 fusion protein. The 2H7 scFv IgAH IgA-T4 fusion polynucleotide sequence (SEQ ID NO: __) is fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO: __), such that when the fusion protein is expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein. cDNA encoding the 2H7 scFv IgAH IgA-T4-CD80 fusion protein (SEQ ID NO: __) is inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The 2H7 scFv IgAH IgA-T4-CD80 cDNA is inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence. The retroviral construct is transfected into Reh, an acute lymphocytic leukemia cell line (ATCC CRL-8286). Transfected cells are screened to select clones that are expressing 2H7 scFv-Ig fusion proteins on the cell surface.

EXAMPLE 25

CHARACTERIZATION OF AN ANTI-4-1BB scFV Ig-CD80 FUSION PROTEIN EXPRESSED ON THE CELL SURFACE OF TUMOR CELLS AND GROWTH OF THE TUMOR CELLS *In Vivo*

This example describes construction of an anti-murine 4-1BB (CD137) scFv fusion protein that has an IgG wild type hinge and CH2 and CH3 domains that is fused to the CD80 transmembrane and cytoplasmic domains. The Example also illustrates the effect of the cell surface expression of the anti-4-1BB scFv IgG CD80 polypeptide when the transfected tumor cells are transplanted into mice.

The heavy and light chain variable regions of a rat anti-4-1BB (CD137) monoclonal antibody (1D8) were cloned, and a single chain Fv construct was prepared essentially as described in Example 1. The heavy chain and light chain variable regions of each antibody were cloned according to standard methods for cloning immunoglobulin genes and as described in Example 1. A single chain Fv construct was prepared as described in Example 1 by inserting a nucleotide sequence encoding a (gly₄ser)₃ peptide linker between the VL region nucleotide sequence of 1D8 (SEQ ID NO: __) and the VH region

nucleotide sequence of 1D8 (SEQ ID NO:___). The polypeptide sequence for 1D8 VL is shown in SEQ ID NO:___, and the polypeptide sequence for the VH domain is shown in SEQ ID NO:___ . The scFv polynucleotide (SEQ ID NO:___) was then fused to human IgG1 wild-type hinge-CH2-CH3 domains according to the methods described in Example 1. The scFv IgG1 fusion polynucleotide sequence was then fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO:___) essentially as described in Example 12, such that when the fusion protein was expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein. cDNA encoding the scFv-IgG-CD80 fusion protein (SEQ ID NO:___) was inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The scFv-Ig-CD80 cDNA was inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence.

The retroviral constructs were transfected into the metastatic M2 clone of K1735, a melanoma cell line, provided by Dr. I. Hellstrom, PNRI, Seattle, WA. Transfected cells were screened to select clones that were expressing scFv-Ig fusion proteins on the cell surface. To demonstrate that the 1D8 scFv IgG-CD80 construct was expressed on the cell surface of the tumor cells, the transfected cells were analyzed by flow immunocytometry. Transfected cells (K1735-1D8) were incubated for one hour on ice in phycoerythrin-conjugated F(ab')₂ goat anti-human IgG. The unbound conjugate was then removed by washing the cells and flow cytometry analysis was performed using a Coulter Epics XL cell sorter. Results are presented in Figure 41A.

The growth of K1735-1D8 transfected cells was examined *in vivo*. K1735-WT cells grew progressively when transplanted subcutaneously (s.c.) in naïve C3H mice. Although the same dose of K1735-1D8 cells initially formed tumors of an approximately 30 mm² surface area, the tumors started to regress around day 7 and had disappeared by day 20 as shown in Figure 41B. Tumor cells that were transfected with a similarly constructed vector encoding a non-binding scFv, a human anti-CD28 scFv construct, grew as well as tumor cells that had not been transfected. The presence of a foreign protein, that is, human IgG1 constant domains or rat variable regions, did not make transfected K1735-WT cells

immunogenic; the growth of the K1735-1D8 cells in C3H mice was identical to that of K1735-WT cells (untransfected).

To investigate the roles of CD4⁺ and CD8⁺ T lymphocytes and NK cells in the regression of K1735-1D8 tumors, naïve mice were injected intraperitoneally (i.p.) with monoclonal antibodies (monoclonal antibodies, typically 50 µg in a volume 0.1 ml) to remove CD8⁺, CD4⁺ or both CD4⁺ and CD8⁺ T cells, or were injected with anti-asialo-GM1 rabbit antibodies to remove NK cells. Twelve days later, when flow cytometry analysis of spleen cells from identically treated mice showed that the targeted T cell populations were depleted, K1735-1D8 cells were transplanted s.c to each T cell-depleted group. K1735-1D8 had similar growth kinetics in mice that had been injected with the anti-CD8 MAb or control rat IgG while removal of CD4⁺ T cells resulted in the growth of K1735-1D8 with the same kinetics as K1735-WT. This failure to inhibit tumor growth after CD4⁺ T cell removal was observed regardless of the presence or absence of CD8⁺ T cells. K1735-1D8 grew in all NK-depleted mice, although more slowly than in the CD4-depleted group. The results are presented in Figure 41C.

EXAMPLE 26

THERAPEUTIC EFFECT OF TUMOR CELLS EXPRESSING ANTI-4-1BB scFv IgG-CD80

FUSION PROTEIN

This Example examines the ability of K1735-1D8 transfected tumor cells expressing an anti-CD137 scFv on the cell surface to generate a sufficient immune response in mice to mediate rejection of established, untransfected wild type tumors. C3H mice were transplanted with K1735-WT tumors (2x10⁶ cells/animal) and grown for approximately six days. Experiments were performed using mice with established K1735-WT tumors of 30 mm² surface area. Mice were vaccinated by s.c. injection of K1735-1D8 or irradiated K1735-WT cells on the contralateral side. Identical injections were repeated at the time points indicated in Figure 42. One group of animals was given four weekly injections of K1735-1D8 cells. According to the same schedule, another group was given irradiated (12,000 rads) K1735-WT cells, and a third group was injected with PBS. The data are

plotted in Figure 42. The WT tumors grew progressively in all control mice and in all mice that received irradiated K1735-WT cells. In contrast, the tumors regressed in 4 of the 5 mice treated by immunization with K1735-1D8. The animals remained tumor-free and without signs of toxicity when the experiment was terminated 3 months later. In the fifth mouse, the tumor nodule decreased in size as long as the mouse received K1735-1D8 cells, but the tumor grew back after therapy was terminated.

In another experiment with 5 mice/group, mice were injected intravenously (i.v.) with 3×10^5 K1735-WT cells to initiate lung metastases. Three days later, K1735-1D8 cells were transplanted s.c. This procedure was repeated once weekly for a month; control mice were injected with PBS. The experiment was terminated when one mouse in the control group died, 37 days after receiving the K1735-WT cells. At that time, lungs of the control mice each had >500 metastatic foci. In contrast, less than 10 metastatic foci were present in the lungs of the immunized mice.

In a third experiment, mixtures of K1735-WT cells and K1735-1D8 cells were injected into immunocompetent syngeneic C3H mice. Mice were injected subcutaneously with K1735-WT cells alone or with a mixture of 2×10^6 K1735-WT cells and 2×10^5 K1735-1D8 cells. Tumor growth was monitored at 5-day intervals.

EXAMPLE 27

EXPRESSION OF ANTI-4-1BB scFv IgG-CD80 FUSION PROTEIN ON THE CELL SURFACE OF SARCOMA CELLS

This Example demonstrates expression of an anti-CD137 scFv on the cell surface of a second type of tumor cell by transfecting a murine sarcoma cell line with an anti-CD137 scFv IgG-CD80 construct.

The 1D8 scFv IgG WTH WTCH2CH3-CD80 polynucleotide (SEQ ID NO:) was transferred from the pLNCX vector into pCDNA3-hygro vector using restriction enzyme digestion and ligation steps according to standard molecular biology methods. The construct was cut with HindIII + ClaI and the scFv fragment was filled in with Klenow (Roche) and the blunt-ended fragment was ligated into EcoRI site of pCDNA3. Ag104 murine sarcoma tumor cells were transfected with the pCDNA3-hygro vector

containing the 1D8 scFv IgG CD80 fusion protein. Hygromycin-resistant clones were screened by flow cytometry using a FITC anti-human IgG antibody to detect expression of the transgene. Only approximately 15% of the resistant clones had detectable fusion protein initially. Positive cells identified by flow cytometry were repeatedly panned on flasks
5 coated with immobilized anti-human IgG (10 μ g/ml) according to standard methods. Panning was performed by incubating cells on the coated plates for 30 min at 37C; the plates were then washed 2-3x in versene or PBS. After each round, cells were tested for IgG expression by FACS. The histogram in Figure 44 shows the staining pattern after four rounds of panning against anti-human IgG (black). Untransfected cells were stained and are
10 indicated in gray. All of the cells in the population were positive.

EXAMPLE 28

CONSTRUCTION AND CHARACTERIZATION OF A BISPECIFIC scFv Ig FUSION PROTEIN AND scFv Ig FUSION PROTEINS WITH A MUTATION IN THE IgG1 CH2 DOMAIN

An anti-CD20 (2H7) scFv IgG fusion protein- was constructed that had a
15 mutant hinge (MT (SSS)) and a mutant CH2 domain in which the proline at residue (position number 238 according to Ward *et al.*, *supra*) was substituted with a serine. The 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 encoding polynucleotide (SEQ ID NO:__) was constructed essentially according to methods described in Examples 1, 5, and 13. The IgG mutant hinge-mutant CH2-wild type CH3 domains were also fused to an anti-CD20 (2H7)-
20 anti-CD40 (40.2.220) bispecific scFv. The anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 encoding polynucleotide sequence is shown in SEQ ID NO:___ and the encoded polypeptide is shown in SEQ ID NO:___.

COS cells were transiently transfected with vectors comprising the polynucleotide sequences encoding 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (SEQ ID
25 NO:__); anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 (SEQ ID NO:__); 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:__); and 2H7 scFv IgAH IgG WTCH2CH3 (SEQ ID NO:___) as described in Example 10. Culture supernatants were collected and the fusion proteins were purified by protein A chromatography (see Example 10). The purified polypeptides were fractionated by SDS-PAGE according to the method

described in Example 10. Rituximab (anti-CD20 monoclonal antibody), and Bio-Rad prestained molecular weight standards (Bio-Rad, Hercules, CA), and Multimark® molecular weight standards (Invitrogen Life Technologies) were also applied to the gel. The results are presented in Figure 45.

5 The 2H7 scFv Ig fusion protein that contains a mutation in the CH2 domain was compared to fusion proteins that have the wild type CH2 domain in an ADCC assay. The assays were performed essentially as described in Examples 11 and 19. Fresh resting PBMC (effector cells) were added to ⁵¹Cr-labeled BJAB cells (target cells) at the ratios indicated in Figure 46. Purified 2H7 scFv IgG MTH (SSS) MTCH2WTCH3, 2H7 scFv IgG
10 MTH (SSS) WTCH2CH3, 2H7 scFv IgAH IgG WTCH2CH3, and Rituximab, each at 10 µg/ml were added to the effector/target cell mixtures and incubated for five hours at 37 °C. Supernatants were harvested and the amount of chromium released was determined as described in Examples 11 and 19. Percent specific killing by each fusion protein is presented in Figure 46.

15

EXAMPLE 29

TUMOR CELL SURFACE EXPRESSION OF AN ANTI-HUMAN CD3 scFv IgG FUSION PROTEIN

An anti-human CD3 scFv Ig CD80 fusion protein was prepared essentially as described in Examples 1 and 12. The fusion protein comprised an anti-human CD3 scFv
20 fused to wild type IgG1 hinge (SEQ ID NO:__) and wild type CH2 (SEQ ID NO:__) and CH3 (SEQ ID NO:__) domains, fused to CD80 transmembrane and cytoplasmic domains (SEQ ID NO:__) to enable cell surface expression of the anti-CD3 scFv. The anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 polynucleotide (SEQ ID NO:__) encoding the polypeptide (SEQ ID NO:__) was transfected in Reh cells and into T51 cells
25 (lymphoblastoid cell line). Expression of the anti-human CD3 scFv IgG fusion protein was detected by flow cytometry using FITC conjugated goat anti-human IgG (see methods in Examples 4, 10, 16, 18). Figure 47A illustrates expression of the anti-human CD3 fusion protein on the cell surface of Reh cells, and figure 47B shows expression of the fusion protein on T41 cells.

ADCC assays were performed with the transfected Reh and T51 cells to determine if expression of the scFv-Ig polypeptides on the cell surface augmented effector cell function. Untransfected and transfected Reh cells and untransfected and transfected T51 cells were pre-labeled with ^{51}Cr (100 μCi) (Amersham) for two hours at 37 °C. Human PBMC served as effector cells and were added to the target cells (5×10^4 cells per well of 96 well plate) at ratios of 20:1, 10:1, 5:1, and 2.5:1. After four hours at 37 °C, culture supernatants were harvested and analyzed as described in Examples 11 and 12. Percent specific killing was calculated as described in Example 12. The results are presented in Figure 48.

EXAMPLE 30

INDUCTION OF CYTOKINE EXPRESSION IN TUMOR CELLS EXPRESSING

ANTI-CD28 scFv ON THE CELL SURFACE

This Example describes the effect of cell surface expressed scFv on cytokine mRNA induction in stimulated lymphocytes co-cultured with tumor cells transfected with an anti-human CD28 scFv IgG-CD80 fusion protein.

Real time PCR analysis was performed on RNA samples from human PBMC stimulated with Reh, Reh-anti-CD28 (2e12) (see Example 12 for construction of 2e12 scFv IgG WTH WHTCH3CH2-CD80 and transfection of Reh cells), and Reh-CD80 (see Example 14) in order to measure the effects of the surface expressed scFv on cytokine production by the PBMC effector cells. For the real-time PCR assay, SYBR Green (QIAGEN) (Morrison *et al.*, *Biotechniques* 24:954-8, 960, 962 (1998)) was used and measured by an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) that measures the formation of PCR product after each amplification cycle. Cells were harvested from cultures and total RNA prepared using QIAGEN RNA kits, including a QIA shredder column purification system to homogenize cell lysates, and RNeasy® mini-columns for purification of RNA. cDNA was reverse transcribed using equal amounts of RNA from each cell type and Superscript II Reverse Transcriptase (Life Technologies). SYBR Green real-time PCR analysis was then performed using the prepared cDNA as template and primer pairs specific for cytokine gene products. The average length

of the PCR products that were amplified ranged from 150-250 base pairs. The cDNA levels for many activation response molecules including IFN γ , TNF α , GM-CSF, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, ICOSL, CD80 and CD86 were assayed. Control reference cDNAs for constitutively expressed genes, including GAPDH, β -actin, and CD3 ϵ were measured in each assay. The most significant induction of specific mRNA was observed for IFN- γ , and more modest induction was observed for CTLA-4 and ICOS.

EXAMPLE 31

CLONING OF AN ANTI-HUMAN 4-1BB ANTIBODY AND CONSTRUCTION OF AN ANTI-HUMAN 4-1BB scFv IG FUSION PROTEIN

10 A hybridoma cell line expressing a mouse anti-human monoclonal antibody (designated 5B9) was obtained from Dr. Robert Mittler, Emory University Vaccine Center, Atlanta, GA. The variable heavy and light chain regions were cloned according to known methods for cloning of immunoglobulin genes and as described herein. Cells were grown in IMDM/15% FBS (Invitrogen Life Technologies) media for several days. Cells in logarithmic growth were harvested from cultures and total RNA prepared using QIAGEN RNA kits, including a QIA shredder column purification system to homogenize cell lysates, and RNeasy® mini-columns for purification of RNA according to manufacturer's instructions. cDNA was reverse transcribed using random hexamer primers and Superscript II Reverse Transcriptase (Invitrogen Life Technologies).

20 cDNA was anchor-tailed using terminal transferase and dGTP. PCR was then performed using an anchor-tail complementary primer and a primer that annealed specifically to the antisense strand of the constant region of either mouse Ck (for amplification of VL) or the appropriate isotype mouse CH1 (for amplification of VH). The amplified variable region fragments were TOPO® cloned (Invitrogen Life Technologies), and clones with inserts of the correct size were then sequenced. Consensus sequence for each variable domain was determined from sequence of at least four independent clones. The 5B9 VL and VH polynucleotide sequences are shown in SEQ ID NOs:___ and ___, respectively, and the deduced amino acid sequences are shown in SEQ ID NOs:___ and ___. The scFv was constructed by a sewing PCR method using overlapping primers containing a

synthetic (gly₄ser)₃ linker domain inserted between the light and heavy chain variable regions (see Example 1). The 5B9 scFv polypeptide (SEQ ID NO:__) is encoded by the polynucleotide sequence comprising SEQ ID NO:___.

5B9 scFv polynucleotide sequence was fused in frame to the polynucleotide sequence encoding the human IgG1 mutant hinge and wild type CH2 and CH3 (MTH (SSS) WTCH2CH3, SEQ ID NO:___) according to methods described in Examples 5, 10, and 13. COS cells were transiently transfected with a vector comprising the 5B9 scFv IgG MTH (SSS) WTCH2CH3 polynucleotide sequence (SEQ ID NO:___). Supernatant was collected and binding of the 5B9 scFv IgG MTH (SSS) WTCH2CH3 polypeptide (SEQ ID NO:___) was measured by flow immunocytofluorimetry essentially as described in Examples 4, 10, 16, and 18. Culture supernatant from the 5B9 hybridoma cell line was also included in the binding assay. Fresh human PBMC were incubated in the presence of immobilized anti-CD3 for four days prior to the binding experiment to induce expression of CD137 on the surface of activated T cells. Stimulated PBMC were washed and incubated with COS or hybridoma culture supernatant containing the 5B9 scFv IgG fusion protein or 5B9 murine monoclonal antibody, respectively, for 1 hour on ice. Binding of 5B9 scFv IgG fusion protein or 5B9 murine monoclonal antibody was detected with FITC conjugated anti-human IgG or anti-mouse IgG, respectively. The results are presented in Figure 49.

EXAMPLE 32

20 CONSTRUCTION OF 2H7 scFv IgG FUSION PROTEINS WITH HINGE MUTATIONS

A 2H7 scFv IgG fusion proteins are constructed with the first cysteine residue and the second cystein in the IgG1 hinge region substituted with a serine residue to provide MTH (SCC) and MTH (CSC). The template for introduction of the mutations is a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO:___). The oligonucleotide introducing the mutations are 5' PCR primer oligonucleotides HIgGMHcys1 (SEQ ID NO:___) and HIgGMHcys2 (SEQ ID NO:___). The constructs are prepared as described in SEQ ID NO:___). The encoding polynucleotides of the mutants are presented in SEQ ID NOs:___) and the polypeptide sequences are provided in SEQ ID NO:___).

Example 33

CONSTRUCTION OF 2H7 V_HL11S scFv (SSS-S) H WCH2 WCH3

A change from leucine to serine at position 11 in the heavy chain variable region (numbering according to Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)) was introduced into the 2H7 scFv MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO:___). The wild type leucine residue was substituted with serine by site-directed mutagenesis using the oligonucleotide Vhser11: 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (SEQ ID NO:___). The 3'-primer for PCR was huIgG1-3' having the sequence 5'-gtc tct aga cta tca ttg acc cgg aga cag-3' (SEQ ID NO:___) (XbaI site underlined and italicized). After PCR amplification, the fragments were inserted into the TOPO® cloning vector and sequenced to confirm the presence of the VH11 leucine to serine mutation. The 2H7 scFv-IgG (SSS-S) H WCH2 WCH3 encoding DNA was shuttled into the PSL1180 cloning vector (Pharmacia Biotech, Inc., Piscataway, NJ). The construct PSL1180-2H7 scFv-IgG (SSS-S) H WCH2 WCH3 was digested with Sac and XbaI to remove the wild type VH domain and the connecting region and CH2 and CH3 domains. The PCR product comprising the VH11 mutant was digested with Sac and XbaI and then inserted into the digested PSL1180 construct according to standard molecular biology procedures. The construct was then digested with Hind III and XbaI, and inserted into the mammalian expression vector pD18 (see methods described in Example 1 and Example 10). The mutant is designated 2H7 scFv VH L11S IgG (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 34

EXPRESSION AND OF 2H7 scFv VH L11S (SSS-S) H WCH2 WCH3

IN STABLE CHO LINES

CHO DG44 cells were transfected by electroporation with approximately 150 micrograms of linearized expression plasmid encoding the 2H7 VH L11S scFv (SSS-S) H WCH2 WCH3. Cultures were plated in selection media containing 100 nM methotrexate, in

96 well, flat bottom tissue culture plates at various numbers of cells/well, ranging from 125 to 2000. Methotrexate resistant clones were selected and culture supernatants were screened for the highest expressors of the fusion protein using a CD20CHO binding assay similar to that described for Figure 1. Clones were amplified after the initial selection in gradually increasing doses of methotrexate. Cells were passaged for two passages in the higher concentration prior to adjusting the concentration to the next higher dose. Clones were amplified to a final concentration of 1 micromolar methotrexate.

Figure 50B illustrates the production levels of 2H7 VH L11S scFv (SSS-S)H WCH2 WCH3. Spent supernatants from amplified CHO cells expressing this molecule and growing in stationary T25 flasks were tested for quantitative binding to CD20 CHO cells by flow cytometry. The activity was converted to protein concentration by generation of a standard curve using the same molecule purified from supernatants with Protein A affinity chromatography (Figure 50A). The concentration of the purified protein was determined by A280 using an extinction coefficient provided by the amino acid composition of the recombinant protein (Vector NTI). Although levels of production varied between clones tested, multiple clones produced over 1 mg/ml. This level of protein expression is over 10-fold higher than the identical molecule except for the amino acid change in V_H.

Figure 51 illustrates the production levels of 2H7 VH L11S scFv (SSS-S) H WCH2 WCH3 by semi-quantitative analysis on SDS-PAGE. Ten microliters of spent supernatant from amplified CHO cells expressing this molecule and growing in stationary T25 flasks were mixed with 10 microliters 2X non-reducing SDS sample buffer, run on SDS-PAGE gels, and stained with coomassie blue.

EXAMPLE 35

CONSTRUCTION AND BINDING CAPACITY OF G28-1 scFv Ig CONSTRUCTS

Contrstruction of the G28-1 (anti-CD37) scFv was performed using total RNA isolated from the G28-1 hybridoma using Trizol (Invitrogen) reagent according to manufacturer's instructions. cDNA was prepared using random primers and the protocol described previously for 2H7 cloning in Example 1. The variable domains of the scFv was cloned using one of two methods: the first method used a family of degenerate 5'

oligonucleotides specific for each V region gene family and a single 3' primer specific for the constant region of either the light or heavy chain using methods and primers described in (Ig-Prime Kit Mouse Ig-Primer Set, Novagen). The second approach used the anchor-tailing methods and primers described in (Gilliland LK et al, Tissue Antigens 47: 1-20 (1995)). In either case, PCR amplified products were cloned into the TOPO cloning vector (Invitrogen). The clones were digested with EcoRI and screened for inserts of the proper size. Positive clones were sequenced as previously described in Example 1.

Specific primers were then designed for each V region, one with the leader sequence and one without. Primers were also designed to include desired linkers and/or restriction sites at the primer ends. PCR reactions were performed on the TOPO cloned DNA using a 25 cycle program with the following profile: 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, followed by a final extension at 72C for 8 minutes. PCR products were gel purified and fragments recovered using a QIAQUICK gel extraction kit (QIAGEN, Valencia, CA). Fragments were diluted 1:50 and 1 microliter used for SEWING PCR reactions according to the methods described in Example 1. The following oligonucleotides were used for the secondary PCR reactions of the V_L domain for the G28-1 scFv:

5' primers with SalI site without leader: 5'-GTTGTTGTGCGACATCCAGATGACTCAGTC TCCA-3' (SEQ ID NO__)

5' primer with HindIII site and leader sequence: 5'-

GTCAAGCTTGCCGCCATGGTATCCA
CAGCTCAGTTCCTTGG-3' (SEQ ID NO__)

3' primer: 5'-

GCCACCCGACCCACCAACCGCCCGAGCCACCGCCACCTTTGATCTCCA
GTTCGGTG CC-3' (SEQ ID NO__)

The primers used for the V_H domain are shown below:

5' sense primer:

TCGGGCGGTGGTGGGTGCGGTGGCGGCGGATCGTCAGCGGTCCAGCTGCA
GCAGTCTGGA-3' (SEQ ID NO__)

3' antisense primer with BclI site: 5'-

TCAGTGCTGATCAGAAGAGACGGTGACTGAGGTTCCTTG-3' (SEQ ID NO__).

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the G28-1 scFv by site-directed mutagenesis. The wild type form of the G28-1 scFv was initially constructed by sewing/overlap PCR to insert a (gly4ser)3 linker between the VL and VH domains as described above. However, no Sac I site was introduced as a part of this fusion of the variable domains, so alternative, nearby restriction sites (HaeII and PvuII) near leucine 11 were used to synthesize the VL+mutated VH domain. Primers were designed to contain one of these sites and the DNA sequence including the L to S change, followed by 12 wild type base pairs. Several attempts at this strategy failed, so an alternative strategy using the Genetailor (Invitrogen) method of site directed mutagenesis was used to introduce the desired mutation. The mutagenesis was carried out according to manufacturer's instructions. Briefly the procedure involves methylation of the plasmid DNA with DNA methylase, amplification of the DNA in a mutagenesis reaction with two overlapping primers, one of which contains the target mutations, transformation of the plasmid into wild type E.coli which digests all methylated DNA and leaves only the unmethylated, mutated amplification product. Both primers are approximately 30 nucleotides in length (not including the mutation site on the mutagenic primer, with an overlapping region at the 5' ends of 15-20 nucleotides, for efficient end-joining of the mutagenesis product. The template for the mutagenesis reaction was 100 ng of a plasmid containing the wild type G28-1 scFvIg construct, and the primers used for the G28-1 VH mutagenesis are as follows:
Forward primer:

5'-GCAGCAGTCTGGACCTGAGTCGGAAAAGCCTG-3' (SEQ ID NO__)

Reverse Primer:

5'-CTCAGGTCCAGACTGCTGCAGCTGGACCGC-3' (SEQ ID NO__)

PCR reactions were performed using the 15 ng methylated template, the primers above, and the usual reaction components as previously described. A 20 cycle program with the following profile was used for amplification: 94C, 30 sec; 55C, 30 sec; 68C, 8 min, followed by a final 68C extension step for 10 minutes. PCR products were transformed into wild type bacteria, and colonies screened by sequencing. Clones with only the desired mutation were isolated and plasmid prepared as previously described Example

33. The mutant is designated G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

5 The expression level of G28-1 fusion proteins was confirmed using Immunoblot analysis according to the methods described in Example 17. Figure 53 illustrates a large increase in protein expression in the VH L11S mutant G28-1 fusion proteins compared to the G28-1 fusion protein without the mutation.

10 The G28-1 scFv Ig fusion proteins were transiently transfected and expressed in COS cells according to methods described in Example 10. Figure 52 illustrates the capacity of the G28-1 scFv Ig fusion proteins from the COS supernatants to bind CD37. Ramos and BJAB cells both express human CD37, and were therefore used to screen the G28-1 supernatants for functional activity. Binding of G28-1 scFv (SSS-S) H WCH2 WCH3 and G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 to CD37+ Ramos cells was measured by flow cytometry according to methods described in Example 2. Each point on the graph represents the mean of five replicate transfections. The graph illustrates that the G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 is able to bind CD37+ Ramos cells.

20 Addition constructs were made with different connecting regions. The pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 vector was digested with BclI and XbaI to remove the connecting region, CH2 and CH3. These were replaced with each different connecting region, CH2 and CH3 according to the methods described in Example 13. The new constructs were designated: G28-1 scFv VHL11S (CSS-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (CSC-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (CSS-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (SSC-P) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (SCS-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (CCS-P) H WH2 WH3 (SEQ ID NO__), and G28-1 scFv VHL11S (SCC-P) H WH2 WH3 (SEQ ID NO__).

25 The G28-1 scFv was also attached to an IgA connecting region, CH2, CH3 and an IgE CH2, CH3, CH4. The pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 plasmid was digested using methods above to remove the connecting region CH2, and CH3. The IgA regions were inserted using methods described in Example 13. The construct was

designated G28-1 scFv VHL11S IgAH IgACH2 T4CH3 (SEQ ID NO__). The IgE CH2 CH3 CH4 region was inserted into the digested pD18 vector above using methods described in Example 39. The construct was designated G28-1 scFv VHL11S IgECH2 CH3 CH4 (SEQ ID NO__).

5

EXAMPLE 36

Characterization of 2H7 scFv Ig Mutant Fusion Proteins

Figure 54 illustrates the binding capacity of purified 2H7 scFv Ig constructs to CD20+ CHO cells. The proteins were transfected into stable CHO cells according to methods described in Example 2. Binding was determined using flow cytometry according to the methods described in Example 2. The graph in Figure 54 illustrates that these proteins retain binding function to CD20 with altered connecting regions. Comparative results were obtained in each of the 2H7 scFv VHL11S mutants with each type of altered connecting region (results omitted).

The ability of 2H7scFv-Ig constructs with mutated connecting regions to kill CD20 positive cells in the presence of peripheral blood mononuclear cells (PBMC) through ADCC was tested by measuring the release of ⁵¹Cr from labeled BJAB cells in a 4 hr. assay using 100:1 ratio of PBMC to BJAB cells. The results shown in Figure 55 indicate that 2H7scFv-Ig mutants can mediate antibody dependent cellular cytotoxicity (ADCC), since the release of ⁵¹Cr was significantly higher in the presence of both PBMC and 2H7scFv-Ig than in the presence of either PBMC or 2H7scFv-Ig alone. Comparative results were obtained in each of the 2H7 scFv VHL11S mutants with each type of altered connecting region (results omitted).

The ability of 2H7scFv-Ig mutant fusion proteins to kill CD20 positive cells in the presence of complement was tested using B cell lines Ramos target cells. Rabbit complement was purchased from Pel-Freez (Rogers, AK), and was used in the assay at a final concentration of 1/10. Purified 2H7scFv-Ig was incubated with B cells and complement for 45 minutes at 37°C, followed by counting of live and dead cells by trypan blue exclusion. The results in Figure 56 show that 2H7scFv-Ig mutants in the presence of rabbit complement, lysed B cells expressing CD20.

EXAMPLE 37
COMPARATIVE BINDING OF IGA, IGG, AND IGE 2H7 scFv CONSTRUCTS

Binding capacity of Ig constructs IgA, IgG and IgE were measured using flow cytometry according to the methods described in Example 2, using a commercially available (Caltag) second step specific for each Ig tail. The results in Figure 57 show that all the IgE constructs were able to bind CD20+ CHO cells comparable to the binding abilities of IgG and IgA. These results also demonstrate that the IgE constructs were detected with the IgE second step, but not the IgA or IgG second step.

EXAMPLE 38
CONSTRUCTION AND CHARACTERIZATION OF 2H7 VH L11S IGE CONSTRUCTS

IgE tail RNA was isolated from SKO-007 cells(ATCC) using QIAGEN QIAshredder homogenization and RNA minikits. Random-primed cDNA was generated according to the usual protocol, with 4 microliters RNA eluted from the QIAGEN columns. Human IgE from the beginning of CH1 through CH4 (approximately 1.2 kb) was isolated by PCR amplification of 5 microliters cDNA, with an amplification profile of 94C, 60 sec; 72C, 2 minutes for 35 cycles, and the following primers:

5' primer: 5'-ggatccacccgctgctgcaaaaacattccctccaatgccacctccgtgac-3' (SEQ ID NO__)

3' primer: 5'-tcatttaccgggatttacagacaccgctcgtctggacggtctgtgaggggctcgtgc-3' (SEQ ID NO__)

PCR fragments were ligated into PCR 2.1-TOPO vector, and transformants screened for inserts of the correct size by digestion with EcoRI according to the methods described in Example 1. One of the clones with the correct sequence from was used as template to amplify the CH2-CH4 domains with appropriate restriction sites attached for subcloning as soluble or cell surface (ORF) forms. The following primers were used with an amplification profile of 94C, 60 sec; 55C, 60 sec; 72C, 2 min; for 35 cycles to amplify a fragment of approximately 950 bp:

5' primer: (attaches BclI site to 5' end of CH2 domain of IgE)

5'-gttgttgatcacgtctgctccagggacttcacc-3' (SEQ ID NO__)

3' primer: (attaches stop codon and XbaI site to 3' end of CH4 of IgE)

5'-gttgtttctagattatcattaccaggatttacagacaccgctcgtg-3' (SEQ ID NO__)

3' primer: (attaches SfuI and BamHI to 3' end of CH4 without a stop codon)

5'-gttgttttcgaaggatccgctttaccagatttacagacaccgctcgtg-3' (SEQ ID NO__)

The IgE CH2CH3CH4 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 IgECH2CH3CH4. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__. The IgE CH2CH3CH4 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 IgECH2CH3CH4(ORF). The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

The human IgE was also amplified as a fragment missing both CH1 and CH2 domains, with only the CH3 and CH4 domains attached to the human IgG1 hinge. Sequential PCR reactions using overlapping 5' oligonucleotides were used to attach the IgG1 hinge to the CH3 domain of human IgE. Primers for the first step of the PCR reaction:

5' Primer: 5'-actcacacatccccaccgtccccagcatccaacccgagaggggtgagc-3' (SEQ ID NO__)

Primers for the second step of the PCR reaction:

5' primer: 5'-tctgatcaggagcccaaattcttgacaaaactcacacatccccaccg-3' (SEQ ID NO__)

3' primer: 5'-gttgtttctagattatcattaccaggatttacagacaccgctcgtg-3' (SEQ ID NO__)

The PCR product was digested with EcoRI and sequenced according to the methods described in Example 1. Positive clones were inserted into pD18 plasmid containing 2H7 VHL11S scFv (SSS-S) H. The construct was designated 2H7 VHL11S scFv (SSS-S) H IgE CH3CH4. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

Binding capacity of 2H7 scFv VH L11S IgECH2 CH3 CH4, was measured using flow cytometry, essentially according to Example 2. The protein was purified using MEP HyperCel, (Cipergen, Catalog # 12035-010, Lot# 200920/0271) chromatography resin and Hydrophobic charge induction chromatography (HCIC). HCIC absorbent is a high capacity, high selectivity, absorbent designed for capture and purification of monoclonal and polyclonal antibodies from various sources including cell culture supernatants. Columns were packed with a 10 ml bead volume of MEP Hypercel, and equilibrated with PBS, pH

7.4 containing 0.1% NaN₃. Approximately 1 liter of 2H7 scFv VHL11S IgE CH₂CH₃CH₄ CHO culture supernatant was then run over the column. A series of citrate buffers ranging from pH 3-6 were prepared for elution of the fusion protein. The column was washed in PBS. Protein was eluted in fifteen 1 ml fractions at pH6, 5, and 4. A final 15 ml fraction was collected at pH 3.5. Aliquots from each fraction were analyzed for A280 and were also subjected to SDS-PAGE, loading roughly 10 micrograms/well based on the A280 reading. The results of these two analyses indicated that the bulk of the protein did not elute in citrate buffers at the higher pH, but eluted at pH4, and the post elution wash at pH3.5 also contained significant amounts of protein.

The ability of these 2H7 VH L11S IgE purified proteins to bind CD20+ CHO cells was determined using flow cytometry according to the methods described in Example 2 using FITC-conjugated goat-anti-human IgE. Figure 58A illustrates that both purified proteins are able to bind CD20+ CHO cells.

The ability of these 2H7 VH L11S IgE purified proteins to mediate ADCC against BJAB target cells with PBMC effectors was measured according to the methods described in Example 2. Figure 58B illustrates that both proteins were able to mediate ADCC at similar levels.

EXAMPLE 39

CONSTRUCTION AND BINDING CAPACITY OF SCFV VH L11S MUTANTS WITH MOUSE IGA AND IGE TAIL REGIONS

Murine IgA was cloned from murine spleen RNA using essentially the same methods used to clone the human IgE tails in Example 38. The PCR reactions were performed with a 94C 60 sec; 52C 60 sec; 72C 2 min amplification profile for 35 cycles. The PCR primers used to clone CH1-CH4 regions were:

5' primer: 5'-atctgttctcctactactcctcctccacct-3' (SEQ ID NO__)

3' primer: 5'-tcagtagcagatgccatcctcctctgacatgatgacagacagct-3' (SEQ ID NO__)

PCR primers used to delete the CH1 region:

5' primer: 5'-gttgtgatcacatctgttctcctactactcctcctccacct-3' (SEQ ID NO__)

3' primer with a stop codon, XbaI site at end of Ig tail, and the T4 mutation in CH3 region:

5'-gttggttctagattatcaatctccctctgacatgatgacagacac-3' (SEQ ID NO__)

3' primer for the ORF, a SfuI and BamHI sites, and T4 mutation in the CH3 region:

5'-gttcttcgaaggatccgcatctccctctgacatgatgac-3' (SEQ ID NO__)

5 The mouse IgACH2 T4CH3 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv and the IgAH. This construct was designated 2H7 VHL11S scFv IgAH mIgACH2 T4CH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

10 The mouse IgACH2 T4CH3 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted in into a pD18 vector that contains 2H7 VHL11S scFv and IgAH. This construct was designated 2H7 VHL11S scFv IgAH mIgACH2 T4CH3 (ORF). The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

15 Murine IgE was cloned murine IgE La2 (ATCC) RNA essentially according the methods described in Example 38. The PCR reactions were performed with a 94C 60 sec; 52C 60 sec; 72C 2 min amplification profile for 35 cycles. Initial PCR primers used to clone the CH1-CH4:

5' primer: 5'-tctatcaggaaccctcagctctacccttgaagccctg-3' (SEQ ID NO__)

3' primer: 5'-gttggttctagattatcaggatggacggaggagggtgtaccaaggct-3' (SEQ ID NO__)

20 PCR primers to remove the CH1 region:

5' primer: 5'-gttggtgatcacgttcgacctgtcaacatcactgagcccacc-3' (SEQ ID NO__)

3' primer with stop codon and XbaI site:

5'-gttggttctagattatcaggatggacggaggagggtgtaccaaggct-3' (SEQ ID NO__)

25 3' primer ORF, SfuI and Bam HI: 5'-gttggtttcgaaggatccgcggatggacggaggagggtgtta-3' (SEQ ID NO__)

The mouse IgE CH2CH3CH4 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 VHL11S mIgECH2CH3CH4. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

The mouse IgE CH2CH3CH4 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 VHL11S scFv mIgECH2CH3CH4(ORF). The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

Binding capacity of 2H7 VH L11S mIgE and mIgA (with mouse tail regions) to CD20+ CHO cells were also measured by flow cytometry according to the methods described in Example 2 using commercially available IgE or IgA second step reagents (Caltag). Each point in Figure 59 represents the mean of a population with brightness corrected by subtracting the binding of the second step alone. This figure illustrates that these constructs have the ability to bind CD20+ CHO cells.

EXAMPLE 40

HPLC PROFILES OF 2H7 scFv Ig MUTANT FUSION PROTEINS

HPLC analysis of purified 2H7 scFv-Ig mutant fusion proteins with altered connecting and CH3 regions. Each protein was purified by Protein A affinity chromatography from supernatants of transfected COS or CHO cells. Twenty-five to fifty micrograms of each sample was run at 1 ml/min in PBS on a TSK-GEL G3000SW_{XL} 30 cm column (Tosoh Biosep, Stuttgart, Germany). The arrow near the beginning of each profile represents the sample injection point. Gel filtration standards (Bio-Rad) included thyroglobulin (670 kDa), gamma globulin (158 kDa), ovalbumin (44 kDa), myoglobin (12.5 kDa), and vitamin B-12 (1.35 kDa). Standards were run at the beginning and end of each experiment. Migration positions of standards are shown and did not vary between experiments. (Figures 60-62)

EXAMPLE 41

BINDING CAPACITY OF 2H7 VH L11S MUTANT FUSION PROTEINS

Binding effects of the CH3 mutant were compared to the non-mutated CH3 fusion protein. The constructs were transfected into COS cells and purified from the supernatant using protein A column purification techniques described in Example 2. Figure 63 illustrates the differential effects of CH3 mutations on binding using flow cytometry

according to the methods described in Example 2. This figure illustrates that some binding ability is lost when the double point mutation is introduced in the CH3 region.

Binding of fluorescein isothiocyanate (FITC) conjugated 2H7 VH L11S mutant fusion proteins was determined using flow cytometry. A 1 mg/ml solution of FITC was prepared in DMSO. Fusion proteins were dialyzed in pH 9.3 bicarbonate buffer overnight at 4C in a volume of 2 liters. Concentration of the protein was adjusted to 1-5 mg/ml prior to conjugation. A series of Falcon 5 ml tubes was set up with varying FITC to protein ratios, ranging from 15-60, but minimally ratios of 20 and 40. Conjugation reactions were incubated at 37C for 30 minutes protected from light. Fluorescing labeled protein was separated from free fluorescein on a 2 ml Sephadex G-25 column equilibrated with PBS, 0.5 M NaCl, and 1% NaN₃. The fluorescein labeled protein eluted from the column first and was collected in a 5ml tube. The degree of labeling was determined by measuring the absorbance of the diluted conjugate at 280 and 494 nm, and utilizing the formulas provided by technical services at Molecular Probes (Eugene, OR). Data has been corrected for FITC: protein ratio. Figure 64 illustrates that these constructs do not lose binding capacity when conjugated to a fluorescent marker.

Purified 2H7 VH L11S constructs were run on a non-reducing SDS gel according to the methods described in Example 2. The migration patterns are presented in Figure 65.

EXAMPLE 42

CHARACTERIZATION OF 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3 IN Lec13 CHO CELLS

2H7 scFv VH L11S (CSC-S) H WCH2 WCH3 were transiently transfected and expressed in Lec13 CHO cells. Lec13CHO cells were used as the mammalian cell hosts for either the 2H7 scFv VHS11 hIgG1 (CSS-S)H WCH2 WCH3 and (CSC-S)H WCH2 WCH3 expressing plasmids in side-by-side transfections. All transfections were performed in 100 mm tissue culture dishes. Cells were transfected when approximately 90% confluent using lipofectamine 2000 (Invitrogen, Catalog #: 11668-027, 0.75 ml), following manufacturer's instructions. Both cell lines were grown in the presence of serum to promote

and maintain adherence to the cell culture dishes, simplifying transfection manipulations, washes, and supernatant harvests. DNA: lipofectamine complexes were allowed to form in the absence of serum and antibiotics, following the suggested protocol /conditions recorded in the product insert. Culture supernatants were harvested 72 hours after transfection, and then again 72 hours after the first harvest. Fusion protein from the two CHO sources was isolated by protein A purification as previously described and used in CD20 binding and ADCC assays.

The ability of mutated fusion protein to mediate ADCC in CD20 positive cells was determined using the methods described in Example 2. Constructs expressed in Lec13 CHO cells exhibited better binding to CD20 CHO target cells and also showed significantly improved activity in ADCC assays relative to the CHO DG44 derived proteins at equivalent concentrations as illustrated in Figure 67.

EXAMPLE 43

CONSTRUCTION OF HIGH AND LOW AFFINITY CD16 ALLELES

The low(V) and high(F) affinity alleles at position 158 of the human CD16 extracellular domain were cloned from cDNA derived from human PBMC using PCR assay. PCR reactions used random primed cDNA made from PBMC stimulated for 3 days with immobilized anti-CD3 antibody (64.1) prior to harvest. PCR reactions included 2, 4, 6 or 8 microliters of cDNA, each primer at 25 pmol, and an amplification profile of 94C 60 sec; 55C 60 sec; 72C 2 min, for 35 cycles. The PCR primers are listed below:

5' primer - no leader peptide: 5'-GTTGTTACCGGTGCAATGCGGACTGAAGATCTCCC
AAAGGCTGTG-3' (SEQ ID NO__)

3'antisense primer: 5'-

GTTGTTTGATCAGCCAAACCTTGAGTGATGGTGATGTTTCAACA-3' (SEQ ID NO__)

Positive clones were sequenced, and inserted into a vector containing an efficient leader peptide and the (SSS-S)H P238SCH2 WCH3 human IgG tail. Two different versions of the CD16 ED fusion proteins were expressed. The first contained the F158 (high affinity) and the second contained the V158 (low affinity) allele. Constructs were cloned into a (SSS-S)H P238S CH2 WCH3 pD18 plasmid and expressed in COS and CHO cells as

previously described in examples 1 and 10. CHO clones were screened for expression using an IgG sandwich ELISA to determine relative expression levels of the fusion proteins in the culture supernatant using the following protocol: Immulon 1V plates were coated at 4C with 0.4 microgram/ml goat anti-human IgG (mouse Adsorbed), (CalTag, Catalog # H10500) in PBS buffer. Plates were then blocked in PBS/1.5% nonfat milk at 4C overnight. Plates were washed three times in PBS/0.1% Tween 20, then incubated with 100 microliters dilution series from CHO clone culture supernatants at room temperature for 3 hours. Four dilutions per clone were added to successive wells, diluting in 5 fold increments from 1:5 to 1:375. In addition a standard curve was derived using CTLA4 hIgG1 (SSS-S)H P238SCH2 WCH3 as a concentration standard. The dilution series utilized 5 fold dilutions starting at 0.5 micrograms/ml; a second set of 8 wells was used to make a 2-fold dilution series starting at 0.34 micrograms/ml. Plates were washed 3 times in PBS/0.1% Tween 20, and incubated with goat anti-human IgG, conjugated to horseradish peroxidase (GAH IgG-HRP) at 1:5000, in PBS/0.5% BSA for 1 hour. Plates were washed four times with PBS/0.1% Tween 20, then TMB chromagen substrate (BD-Pharmingen) was added for 10 minutes, and reactions stopped by addition of 100 microliters 1N sulfuric acid. Plates were then read at 415nm on a SpectraCount plate reader. Concentrations of fusion protein were estimated by comparison of the ODs in the linear range to the CTLA4Ig standard curve run on each plate.

Proteins were purified using Protein A purification and were directly conjugated to fluorescein isothiocyanate (FITC) as described in Example 42. These proteins were run out on SDS gels under reduced and nonreduced conditions according to the methods described in Example 2. The migration of these proteins is presented in Figure 68.

The ability of the high and low CD16 alleles to bind 2H7 VH111S (CSC-S) H WCH2 WCH3 or bind 2H7 VH111S (SSS-S) H (P238S)CH2 WCH3 expressed on the cell surface of CD20+ CHO target cell is determined using flow cytometry according to the methods described in Example 2. The results in Figure 69 demonstrate both the high and low affinity alleles were able to bind 2H7 VHL11S (CSC-S)H WCH2 WCH3 (SEQ ID NO___) and lost some binding capabilities when the P238S mutation was introduced into the CH2 region of the construct (SEQ ID NO___).

EXAMPLE 44

MAMMALIAN DISPLAY SYSTEM

Figure 70A diagrams how FITC conjugates of FcRIII (CD16) soluble fusion proteins bind to 2H7 scFv-Ig constructs that are attached to CD20 expressed by CHO cells.

- 5 The CD16 binding to a scFv-Ig provides a screening tool for detecting changes in CD16 binding to an altered scFv-Ig constructs containing targeted or site-specific mutations. Changes in CD16 binding properties may be changes in binding of either CD16 high affinity protein (158F) or CD16 low affinity protein (158 V) or both.

- A schematic representation of such a screening process is diagrammed in
10 Figure 70B, where scFv-Ig constructs are displayed on the cell surface of mammalian cells. The scFv-Ig molecules in this example are displayed on the cell surface because they contain a transmembrane domain anchor. These molecules may represent a single scFv-Ig construct or may be introduced into a population of mammalian cells as a library of such molecules. Transfected cells with altered binding properties can then be panned, sorted, or
15 otherwise isolated from other cells by altering the stringency of the selection conditions and using CD16 fusion proteins as the binding probe. Cells that express scFv-Ig molecules with altered binding to either CD16 high affinity allele (158F) or CD16 low affinity allele (158V) or both can be isolated. For example, this display system can be used to create a library of
20 mutated Ig tails with short stretches of CH2 sequence replaced with randomized oligonucleotides or possibly randomization of a single residue with all possible amino acid substitutions, including synthetic amino acids. Once such a library is constructed, it can be transfected into COS cells by methods well known in the art. Transfectants can then be bound to the labeled CD16 constructs, and panned or sorted based on their relative binding properties to multiple allelotypes/isoforms. Panned cells are harvested, and the plasmid
25 DNA is isolated and then transformed into bacteria. This process may be repeated iteratively multiple times until single clones are isolated from the mammalian host cells (see Seed B and Aruffo A, PNAS 84:3365-3369 (1987) and Aruffo A and Seed B, PNAS 84: 8573-8577 (1987)). One such use of this type of screening system would be to isolate Ig tails which bind equally well to both the high and low affinity alleles of CD16 with the goal
30 of improving effector functions mediated by scFv-Ig constructs in multiple subpopulations

of patients. Ig tails with altered binding properties to other Fc receptors can also be selected using the display system described. Other display systems for example those that use bacteriophage or yeast are not suitable for selection of Ig tails with altered FcR binding properties because of the requirement for glycosylation in the Ig CH2 domain that would not occur in non-mammalian systems.

This system is also useful for selection of altered scFv-Ig molecules that will be produced at higher levels. In this example, mammalian cells such as COS cells can be transfected with a library of scFv-Ig constructs in a plasmid that directs their expression to the cell surface. COS cells that express the highest levels of the scFv-Ig molecules can be selected by techniques well known in the art (for example panning, sterile cell sorting, magnetic bead separation, etc), and plasmid DNA is isolated for transformation into bacteria. After several rounds of selection single clones are isolated that encode scFv-Ig molecules capable of high level expression. When the isolated clones are altered to remove the membrane anchor and then expressed in mammalian cells, the scFv-Ig constructs will be secreted into the culture fluid in high levels. This reflects the common requirement of secreted glycoproteins and cell surface glycoproteins for a signal peptide and processing through the golgi for expression, so that selection for a molecule that illustrates an improvement in expression levels on the cell surface will also select for a molecule that illustrates an improvement in levels of secreted protein.

EXAMPLE 45

CHARACTERIZATION OF G28-1 MABS AND SCFVS

Ability of G28-1 mAbs and scFvs to induce apoptosis was measured by binding Annexin V, using the methods described in Example 3. The results in Figure 71 demonstrate that the Annexin V binding of G28-1 antibodies and scFv is increased when treated together with 2H7 antibodies and scFv constructs.

EXAMPLE 46

CONSTRUCTION OF FC2-2 SCFV CONSTRUCTS

Contrstruction of the FC2-2 (anti-CD16) scFv was performed using total RNA isolated from the FC2-2 hybridoma and cloned using methods described in Example

35. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__. The specific primers for the secondary PCR reaction are listed bellow. The following are primers for the light chain variable region:

5 5' primer with HindIII site with no leader: 5'-GTTGTTAAGCTTGCCGCCATGGATTACAGGCCCAGGTTCTT-3' (SEQ ID NO __)

5' primer with SalI site and leader: 5'-GTTGTTGTCGACATTGTGATGTCACAGTCTCCATCCTCCCTA-3' (SEQ ID NO __)

3' primer:

10 5'-TCAGTGCTGATCATGAGGAGACGGTGACTGAGGTTTCCTT-3' (SEQ ID NO __)

The following are primers for the heavy chain variable region:

5' primer: 5'-

TCGGGCGGTGGTGGGTGCGGTGGCGGCGGATCGTCACAGGTGCAGTTGAAGGAGTCAGGA-3' (SEQ ID NO __)

15 3' primer: 5'-

ACCCGACCCACCACCGCCCGAGCCACCGCCACCTTTTATTTCCAGCTTG
GTGCCACCTCCGAA-3' (SEQ ID NO __)

20 A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the FC2-2 scFv by site-directed mutagenesis according to the methods described in Example 33. The scFv was attached to the (SSS-S) H WCH2 WCH3 IgG tail according to methods described in Example 33. The mutant is designated FC2-2 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

25

EXAMPLE 47

CONSTRUCTION OF 5B9 scFv CONSTRUCTS

Contrstruction of the 5B9 (anti-CD137) scFv was performed using total RNA isolated from the 5B9 hybridoma and cloned using methods described in Example 35. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide

sequence is provided in SEQ ID NO:____. The specific primers for the secondary PCR reaction are listed below. The following are primers for the light chain variable region:

5' primer with HindIII site with no leader: 5'-GTTGTTAAGCTTGCCGCCATGAGGTTCTCTGCTCAGCTTCTG-3' (SEQ ID NO__)

5 5' primer with SalI site and leader: 5'-GTTGTTGTCGACATTTGTGATGACGCAGGCTGCATTCTCCAATT-3' (SEQ ID NO__)

3' primer: 5'-TCAGTGCTGATCAGAGGAGGACGGTGACTGAGGTTTCCTTG-3' (SEQ ID NO__)

The following are primers for the heavy chain variable region:

10 5' primer: 5'-
CGGGCGGTGGTGGGTCGGGTGGCGGCGGATCGTCACAGGTGCAGCTGA
AGCAGTCAGGA-3' (SEQ ID NO__)

3' primer: 5'-
CCCGACCCACCACCGCCCGAGCCACCGCCACCCTTCAGCTCCAGCTTG

15 GTGCCAGCACC-3' (SEQ ID NO__)

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced into the 5B9 scFv by site-directed mutagenesis and attached to (SSS-S) H WCH2 WCH3 according to the methods described in Example 33. This construct was designated 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3. The
20 polynucleotide sequence is provided in SEQ ID NO:____, and the encoded polypeptide sequence is provided in SEQ ID NO:____.

EXAMPLE 48

CONSTRUCTION OF UCHL1 scFv CONSTRUCTS

Contrstruction of the UCHL1 (anti-CD45RO) scFv was performed using total
25 RNA isolated from the UCHL1 hybridoma and cloned using methods described in Example 35 The polynucleotide sequence is provided in SEQ ID NO:____, and the encoded polypeptide sequence is provided in SEQ ID NO:____. The following are primers for the light chain variable region:

5' primer with HindIII site: 5'-

GTTGTTAAGCTTGCCGCCATGAAGTTGCCTGTTAGGCTG

TTGGTGCTG-3' (SEQ ID NO__)

3' primer with Sac site: 5'-

5 AGAGCTCCACCTCCTCCAGATCCACCACCGCCCGAGCCAC

CGCCATCTTTGATTTCAGCTTGGT-3' (SEQ ID NO__)

The following are primers for the heavy chain variable region:

5' primer: 5'-

TTTCAGAGTAATCTGAGAGCTCCACCTCCTCCAGATCCACCACCGC

10 CCGA-3' (SEQ ID NO__)

3' primer: 5'-TCAGTGCTGATCATGCAGAGAGACAGTGACCAGAGTCCC-3' (SEQ ID NO__)

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the 5B9 scFv by site-directed mutagenesis and connected to a(SSS-S) HWCH2 WCH3 according to the methods described in Example 33. The mutant is designated UCHL1 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 49

20 L6 VHL11S scFv (SSS-S) H WCH2 WCH3

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the L6 scFv (SSS-S) H WCH2 WCH3 (constructed according to methods described in Example 106) by site-directed mutagenesis according to the methods described in Example 33. The L6scFvIg (SSS-S) H WCH2 WCH3 pD18 plasmid was used as template. Positive clones were inserted into the pD18 plasmid containing (SSS-S) H WCH2 WCH3 according to methods described in Example 33. The mutant is designated L6 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

PCR primers are listed bellow:

5' Primer with PstI restriction site:5'-

ggcggatctctgcagatccagttggtgcagctctggacctgagtcgaagaagcct

ggagag-3' (SEQ ID NO__)

5 3'Primer: 5'-ggacagtgggagtgacc-3' (SEQ ID NO__)

EXAMPLE 50

CONSTRUCTION OF HD37 scFv VHL11S CONSTRUCT

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the HD37 scFv by site-directed
10 mutagenesis according to the methods described in Example 33. The HD37 scFv (SSS-S)H WCH2 WCH3 pD18 plasmid was used as a template. Positive clones were inserted into the pD18 plasmid containing (SSS-S) H WCH2 WCH3 according to methods described in Example 33. The mutant is designated HD37 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide
15 sequence is provided in SEQ ID NO:__. PCR primers are listed bellow:
5' primer: 5'-caggttcagctgcagcagctctggggctgagtcggtgaggcctgg-3' (SEQ ID NO__)
3' primer: 5'-ggaggattcgtctgcagtcagagtggc-3' (SEQ ID NO__)

EXAMPLE 51

2H7 scFv VHL11S CONSTRUCTS

20 Additional 2H7 VHL11S constructs were made with different connecting regions. The pD18 2H7 scFv VHL11S (SSS-S) H WCH2 WCH3 vector was digested with BclI and XbaI to remove the connecting region, CH2 and CH3. Theses were replaced with each different connecting region, CH2 and CH3 according to the methods described in Example 13. The new constructs were designated: 2H7scFv VHL11S (CSS-S) H WH2
25 WH3 (SEQ ID NO__), 2H7scFv VHL11S (CSC-S) H WH2 WH3 (SEQ ID NO__). 2H7 scFv VHL11S was also attached to an IgA connecting region, CH2, CH3 and an IgE CH2, CH3, CH4. The pD18 2H7 scFv VHL11S (SSS-S) H WCH2 WCH3 plasmid was digested using methods above to remove the connecting region CH2, and CH3. The IgA regions were inserted using methods described in Example 13. The construct was designated 2H7

scFv VHL11S IgAH IgACH2 T4CH3 (SEQ ID NO___). The IgE CH2 CH3 CH4 region was inserted into the digested pD18 vector above using methods described in Example 39. The construct was designated 2H7 scFv VHL11S IgECH2 CH3 CH4 (SEQ ID NO___).

EXAMPLE 52

5 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a human IgG1 connecting region, CH2 and CH3 region, where in the second
10 cysteine and the proline in the connecting region have been changed to serines (SSS-S) as described in Example 32. . The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 53

15 2H7 scFv VH L11S IgE CH2 CH3 CH4

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. . The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded
20 polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 54

25 2H7 scFv VH L11S mIgE CH2 CH3 CH4

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the
25 leucine has been changed to a serine, as described in Example 33. This binding region is attached to a mouse IgE constant region containing CH2, CH3 and CH4 as described in Example 39. . The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 55

2H7 scFv VH L11S mIgAH WIGACH2 T4CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 39. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 56

2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322S mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a serine using overlapping PCR assay. An (SSS-S) H WCH2 WCH3 IgG1 template in the pD18 vector was used for PCR amplification, to create (SSS-S) H derivatives containing these CH2 mutations. PCR reactions used a cycling profile of 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, for 37 cycles to complete the reactions. This IgG1 derivative was constructed by using sequential PCR reactions with overlapping oligonucleotides in the primary and secondary reactions. The primary amplification primers introduced the mutation(s), but deleted one end of the Fc domain. Secondary reaction primers reattached these ends using overlapping primers. The first overlapping primer was added at the beginning of the PCR, the reactions allowed to proceed for 12 cycles, paused and then the

second overlapping primer added to the reactions followed by 25 more cycles to complete the overlap extension PCR reactions.

Primers for the first PCR reaction:

5' Primer: 5'-ggagatggttttctcgatgggggctggagggttggagaccgagcactgtactcc-3' (SEQ ID NO)

3' primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO)

PCR products were cloned into TOPO vector and sequenced for verification.

Positive vectors were used as templates for the second overlapping PCR reaction.

5' primer: 5'-ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

5'primer overlapping primer: 5'-

ccccaccgtccccagcacctgaactcctggggggatcgtcagtcttctcttcccc

caaaacc-3' (SEQ ID NO)

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO)

PCR product was cloned into TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 57

2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322S mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a serine using overlapping PCR assay. The mutation in the CH2 region was added by overlapping PCR reaction essentially according to

Example 57, with (CSS-S) H WCH2 WHC3 IgG1 pD18 vector as a template in the first PCR reaction and different primers in the second PCR reaction, which are listed below.

5'primer: 5'- ccgtctctgatcaggaccccaaatcttctgtacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO__)

5 5'overlapping primer: 5'-

tccccaccgtccccagcacctgaactcctggggggatcgctcagttctcttccccccaaaacc-3'

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO)

PCR products were cloned into the TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide

10 sequence is provided in SEQ ID NO:__.

EXAMPLE 58

2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the
15 leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG1 CH2 region and a wild type IgG1 CH3 region. The mutation P331S mutation in the CH2 region, where the proline at residue 331
20 has been changed to a serine, was incorporated using a single PCR reaction, using a (SSS-S) H WCH2 WCH3 pD18 template and a cycling profile of 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, for 37 cycles. The specific primers for reaction are listed below.

5' primer: 5'ccgtctctgatcaggagcccaaatcttctgtacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

25 3' Primers: 5'-
gcagggtgtacacctgtggttctcggggctgccctttggcttggagatggtttctcgatggaggctgggagg-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 59

2H7 scFv VH L11S (CSS-S) H P331S CH2 WCH3

This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The mutation P331S mutation in the CH2 region, where the proline at residue 331 has been changed to a serine, was incorporated using a single PCR reaction, using a (CSS-S) H WCH2 WCH3 pD18 template and a cycling profile of 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, for 37 cycles.

10 The specific primers for reaction are listed below.

5' primer: 5'-ccgtctctgatcaggacccccaaatcttgacaaaactcacatccccaccgtccccagc-3' (SEQ ID NO)

3' Primer: 5'-gcagggtgtacacctgtggttctcggggctgccctttggctttggagatggtttctcgatggaggctgggagg-3' (SEQ ID NO)

15 The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 60

2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3

20 This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The T256N mutation in the CH2 region, the threonine at residue 256 has been changed to an asparagine, using the overlapping PCR methods described in Example 56. The specific primers are listed below.

Primers for the first PCR reaction:

5' primer: 5'-ttcctcttccccccaaaacccaaggacaccctcatgatctcccgaaccctgaggtcac-3' (SEQ ID NO)

3' primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO)

PCR product cloned into TOPO vector and sequenced. This product was used as the template in the second PCR reaction. Primers for the second PCR reaction:

5' primer: 5'-ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

5'overlapping primer: 5'-

tccccaccgtccccagcacctgaactcctggggggatcgctcagtcttctcttccccccaaaacc-3' (SEQ ID NO)

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 61

2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The RTPE/QNAK mutation in the CH2 region, where residues 255-258 have been mutated from arginine, threonine, proline, glutamic acid to glutamine, asparagines, alanine and lysine, respectively, using the overlapping PCR reactions described in Example 56. The specific primers are listed below.

PCR primers for the first PCR reaction:

5' primer: 5'-ttcctcttccccccaaaacccaaggacaccctcatgatctcccagaacgctaagggtcacatgc-3' (SEQ ID NO __)

3' primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO)

The PCR product was cloned into TOPO vector, sequenced and used as a template for the second PCR reaction. The primers for the second PCR reaction:

5 primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

5 5'overlapping primer:5'-

tccccaccgtccccagcacctgaactctgggggatcgtcagtcttcttccccccaaaacc-3' (SEQ ID NO)

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:_____.

10

EXAMPLE 62

2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The K290Q mutation in the CH2 region, where the Lysine at residue 290 has been changed to a Glutamine, using a single PCR reaction according to the methods described in Example 58. The specific primers used in this reaction are listed below.

15 5' primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

20 3' primer: 5'-gctcccgcggtgtgtcttggc-3' (SEQ ID NO)

25 PCR products were cloned into TOPO and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:_____.

EXAMPLE 63

2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The A339P mutation in the CH2 region, where the alanine at residue 339 has been changed to a proline, using a single PCR reaction according to the methods described in Example 58. The specific primers used in this reaction are listed below.

5' primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

3' Primer: 5'-ggaggtgggcagggtgtacacctgtggttctcggggctgccctttgggttggagatgg-3' (SEQ ID NO)

PCR products were cloned into TOPO and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 64

G28-1 scFv (SSS-S) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region made according to methods described in Example 35. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. This construct has previously been referred to as G28-1- MHW TG1C and G28-1 scFv Ig, both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 65

G28-1 scFv IgAH WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region made according to methods described in Example 35. This binding region is connected to a human IgA connecting region and wild type human IgG CH2 and CH3 constant regions as described in Example 5. This construct has previously been referred to as: G28-1-IgAHWTG1C. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 66

G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3

10 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5.

15 This connecting region is attached to wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 67

G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3

20 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according

25 to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 68

G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the second cysteine and the proline has been changed to serine (CSC-S), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 69

G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the first and second cysteines have been changed to serines (SSC-P), according to methods described in Example 13. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 70

CTLA4 (SSS-S) H P238SCH2 WCH3

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced using a PCR assay. PCR reactions were performed using random primed cDNA prepared from human tonsil B cell RNA. PCR

amplifications used an amplification profile of 94C 4 min; [94C 1 min; 55C 1 min; 72C 1min; for 30 cycles followed by a final extension step for 6 minutes at 72C. PCR fragments were TOPO cloned and clones with EcoRI inserts of approximately 800 bp were sequenced as described in Example 1. The primers used for the PCR are listed below:

5 5' primer: 5'-

gttggtgatcaggagcccaaatcttctgacaaaactcacacatctccaccgtccccagcacctgaactcctgggtgg
accgtcagtcttcc-3' (SEQ ID NO)

3' primer: 5'gttggttctagattatcatttaccgagacag-3' (SEQ ID NO)

This construct has previously been referred to as CTLA-4 IgG MTH (SSS)
10 MTCH2WTCH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 71

CTLA4 (CCC-P) WH WCH2 WCH3

15 This construct has a CTLA-4 binding region as described in Example 14. This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. This construct has previously been referred to as CTLA-4 IgG WTH (CCC) WTCH2CH3, which has the same sequence as the
20 above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 72

FC2-2 scFv (SSS-S) H WCH2 WCH3

This construct has a FC2-2 (anti-CD16) single chain Fv made according to
25 methods described in Example 46. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The

polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 73

FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3

5 This construct has a FC2-2 (anti-CD16) single chain Fv with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 46. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting
10 region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 74

UCHL-1 scFv (SSS-S) H WCH2 WCH3

15 This construct has a UCHL-1 (anti-CD45RO) single chain Fv made according to methods described in Example 48. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting
20 region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 75

UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3

25 This construct has a UCHL-1 (anti-CD45RO) single chain Fv with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 48. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting

region is connected to a wild type human IgG1 CH2 and CH3 constant region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 76

5 5B9 scFv (SSS-S) H WCH2 WCH3

This construct has a 5B9 (anti-CD137) single chain Fv made according to methods described in Example 47. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is
10 connected to a wild type human IgG1 CH2 and CH3 constant region as described in Example 1. This construct has previously been referred to as 5B9 scFv IgG MTH (SSS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

15 EXAMPLE 77

5B9 scFv VHL11S (SSS-S) H WCH2 WCH3

This construct has a 5B9 (anti-CD137) single chain Fv with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 47. This binding region is connected to a
20 mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 constant region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

25 EXAMPLE 78

2H7 scFv (SSS-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1

connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type IgG1 CH2 and CH3 constant region as described in Example 1. This construct has previously been referred to as 2H7-MHWTG1C, CytoxB-(MHWTG1C) – Ig, anti-CD20 scFv IgG MTH (SSS) WTCH2CH3, CytoxB-MHWTG1C, 2H7 scFv-human IgG1 wild type hinge-CH2-CH3, and 2H7 scFv IgG MTH (SSS) WTCH2CH3, which all have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 79

2H7 scFv (SSS-S) H P238SCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This construct has previously been referred to as 2H7 scFv IgG MTH (SSS) MTCH2WTCH3, anti-CD20 scFv IgG MTH (SSS) MTCH2CH3, and CytoxB-MHMG1C which all have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 80

2H7 scFv IgAH WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a human IgA connecting region and wild type human IgG1 CH2 and CH3 constant regions as described in Example

5. This construct has previously been referred to as 2H7 scFv IgAH IgG WTCH2CH3, 2H7 scFv IgA hinge-IgG1 CH2-CH3, and CytosB-IgAHWTHG1C, which all have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:_____.

5

EXAMPLE 81

2H7 scFv IgAH WIGACH2 T4CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This construct has previously been referred to as 2H7 scFv IgAH IgAT4, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:_____.

15

EXAMPLE 82

2H7 scFv IgAH WIGACH2 WCH3 + JCHAIN

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a wild type human IgA connecting region as described in Example 5. This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13. This constant region is attached to a J-chain region as described in Example 13. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:_____.

25

EXAMPLE 83

2H7 scFv (CCC-P) WH WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1. This connecting region is attached

to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv Ig WTH (CCC) WTCH2CH3, 2H7 scFv IgG WTH WTCH2CH3, and 2H7 scFv-Ig, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 84

2H7 scFv (SSS-S) H WCH2 F405YCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The F405Y mutation, where the phenylalanine at residue 405 has been changed to a tyrosine, was introduced according to methods described in Example 21. This construct has previously been referred to as 2H7 scFv MTH WTCH2 MTCH3 Y405, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 85

2H7 scFv (SSS-S) H WCH2 F405ACH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The F405A mutation, where the phenylalanine at residue 405 has been changed to an alanine, was introduced according to methods described in Example 21. This construct has previously been referred to as 2H7 scFv MTH WTCH2 MTCH3 A405, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 86

2H7 scFv (SSS-S) H WCH2 Y407ACH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The Y407A mutation, where the tyrosine at residue 407 has been changed to an alanine, was introduced according to methods described in Example 21. This construct has previously been referred to as scFv MTH WTCH2 MTCH3 A407, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 87

2H7 scFv (SSS-S) HWCH2 F405A, Y407ACH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S), according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The F405A and Y407A mutation, where the phenylalanine at residue 405 has been changed to an alanine and the tyrosine at residue 407 has been changed to an alanine, was introduced according to methods described in Example 21. This construct has previously been referred to as scFv MTH WTCH2 MTCH3 A405A407, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 88

2H7 scFv (CSS-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (CSS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

Example 89

2H7 scFv (SCS-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the first and third cysteines have been changed to serines and the proline has been changed to serine (SCS-S), according to methods described in Example13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv IgG MTH (SCS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 90

2H7 scFv (SSC-P) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the first and second cysteines have been changed to serines (SSC-P), according to methods described in Example13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (SSC) WTCH2CH3, which has

the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 91

2H7 scFv (CSC-S) H WCH2 WCH3

5 This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the second cysteine and the proline has been changed to serine (CSC-S), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example

10 1. This construct has previously been referred to as 2H7 scFv MTH (CSC) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 92

2H7 scFv (CCS-P) H WCH2 WCH3

15 This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where third cysteine has been changed to a serine (CCS-P), according to methods described in Example 22. This connecting region is attached to wild type human

20 IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (CCS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 93

2H7 scFv (SCC-P) H WCH2 WCH3

25 This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where first cysteine has been changed to a serine (SCC-P), according to

methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (SCC) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 94

2H7 scFv VH L11S (SSS-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv VH11SER IgG MTH (SSS) WTCH2CH3 and 2H7 scFv VHSER11 WTH WTCH2CH3, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 95

2H7 scFv VH L11S (CSS-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 33. This binding region is attached to a mutant human IgG1 connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

Example 96

G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the first and third cysteines have been changed to serines and the proline has been changed to serine (SCS-S), according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 97

G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where third cysteine has been changed to a serine (CCS-P), according to methods described in Example 22. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 98

G28-1 scFv VH L11S (SCC-P) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where first cysteine has been changed to a serine (SCC-P), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in

Example 1. The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 99

G28-1 scFv VH L11S mIgE CH2 CH3 CH4

5 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a wild type mouse IgE CH2 CH3 and CH4 region using methods described in Example 39. The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded
10 polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 100

G28-1 scFv VH L11S mIgAH WIGACH2 T4CH3

 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the
15 leucine has been changed to a serine as described in Example 35. This binding region is attached to a connecting region from mouse IgA as described in Example 39. This connecting region is attached to a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a 4 amino acid truncation at residues as described in Example 39. The polynucleotide sequence is provided in SEQ ID NO:___, and
20 the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 101

G28-1 scFv VH L11S hIgE CH2 CH3 CH4

 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the
25 leucine has been changed to a serine as described in Example 35. This binding region is attached to a wild type human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 102

G28-1 scFv VH L11S HIGAH WIGACH2 T4CH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 103

HD37 scFv IgAH WCH2 WCH3

The HD37 scFv was cloned from the HD37 hybridoma using the Novagen – Ig family primer sets, TOPO cloning and sequencing and sewing PCR assay. For the initial PCR reactions prior to sewing, the TOPO clone templates HD37 VH C-1 and HD37 KVL B-9 were used at 1:100 with an amplification profile of 94C 30 sec; 55C, 30 sec; 72C, 30 seconds for 25 cycles. To provide templates for the secondary SEWING PCR reactions, primary reaction products were gel purified, QIAQUICK purified, and the eluates diluted 50 fold. One microliter each VL and VH overlapping templates were added to PCR reactions with the following amplification profile: 94C, 60 sec; 55C, 60 sec; 72C, 60 sec; for 30 cycles. After two cycles, the machine was paused, and the flanking 5'VL and 3'VH primers were added to the reactions at 25 pmol each, and the PCR reactions resumed. PCR products were checked on a gel for the presence of an 750-800 bp fragment, and the reactions products QIAQUICK purified and digested with the appropriate restriction enzymes for insertion into pD18 Ig expression vectors.

PCR of VL domain with native leader peptide and part of glyser linker:

5' primer: 5'-gttgtaagcttgccgccatggagacagacacactcctgctatgg-3' (SEQ ID NO)

3' primer: 5'gccacccgacccaccaccgcccagaccgccaccttgattccagcttggtgcctcc-3' (SEQ ID NO)

PCR of VL domain without leader peptide (SalI site) and part of glyser linker:

5' primer: 5'-gttggtgacattgtgctgacccaatctcca-3' (SEQ ID NO)

3' priemr: 5'-gccacccgacccaccaccgcccagccaccgccaccttgattccagcttggtgcctcc-3' (SEQ ID
5 NO)

PCR of VH domain with part of glyser linker and BclI site for fusion to -Ig tails.

5'primer: 5'-tcgggcgggtgggtgggtggcgccggtgatcgacaggttcagctgcagcagctctgg-3' (SEQ ID
NO)

10 3' primer: 5'-tcagtgtgatcagaggagacgggtgactgaggttccttg-3' (SEQ ID NO)

This binding region is connected to a wild type human IgA connecting region and wild type human IgG CH2 and CH3 constant regions as described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as HD37 scFv-
15 IgAHWTG1C and HD37- IgAHWTG1C, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 104

HD37 scFv (SSS-S) H WCH2 WCH3

20 This construct has a HD 37 single chain Fv as described in Example 103. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been
25 referred to as HD37- MHWTG1C and HD37 scFv-IgMHWTG1C, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 105

HD37 scFv VH L11S (SSS-S) H WCH2 WCH3

This construct has a HD 37 single chain Fv with a mutation in the heavy chain variable region at amino acid residue 11, where leucine has been changed to serine according to the methods described in Example 50. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 106

10 L6 scFv IGAH WCH2 WCH3

The L6 scFv was cloned from the L6 hybridoma (I Hellstrom) using the anchor-tailing method described in the Tissue Antigens Paper from 1996. The PCR profile was 94C, 1 min; 50C, 2 min; 72C, 2 min; for 35 cycles. Once consensus sequence was obtained for VL and VH regions from at least 4 TOPO clones, primers were ordered for PCR reactions prior to SEWING PCR reactions as follows: For the initial PCR reactions prior to sewing, the TOPO cloned templates L6VK and L6VH were used at 1:100 with an amplification profile of 94C 30 sec; 55C, 30 sec; 72C, 30 seconds for 25 cycles. To provide templates for the secondary SEWING PCR reactions, primary reaction products were gel purified, QIAQUICK purified, and the eluates diluted 50 fold. One microliter each VL and VH overlapping templates were added to PCR reactions with the following amplification profile: 94C, 60 sec; 55C, 60 sec; 72C, 60 sec; for 30 cycles. After two cycles, the machine was paused, and the flanking 5'VL and 3'VH primers were added to the reactions at 25 pmol each, and the PCR reactions resumed. PCR products were checked on a gel for the presence of an 750-800 bp fragment, and the reactions products QIAQUICK purified and digested with the appropriate restriction enzymes for insertion into pD18 Ig expression vectors.

PCR of VL domain with native leader peptide and part of glyser linker:

L6VLHindIII: 5'-gttgtaagcttgccgcatggatttcaagtcagatttcagcttc-3' (SEQ ID NO)

L6VLLK3: 5'-gccacccgacccaccaccgcccagccaccgcccaccagagagctctttcagctccagcttggt-3'
(SEQ ID NO)

PCR of VL domain without leader peptide (Sall site) and part of glyser
linker:

- 5 5' primer: 5'-gttggtgacattgttctctcccagctccagcaatcctgtctg-3' (SEQ ID NO)
3' primer: 5'-gccacccgacccaccaccgcccagccaccgcccaccagagagctctttcagctccagcttggt-3' (SEQ
ID NO)

PCR of VH domain with part of glyser linker and BclI site for fusion to -Ig
tails.

- 10 5': 5'-tcgggcggtggtgggtcgggtggcggcgatctctgcagatccagttggtgcagtct-3' (SEQ ID NO)
3'Bcl: 5'-tcagtgtgatcagaggagactgtgagagtggcgcttg-3' (SEQ ID NO)

- This binding region is connected to a human IgA connecting region and wild
type human IgG1 CH2 and CH3 constant regions as described in Example 5. This
connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as
15 described in Example 1. This construct has previously been referred to as L6 scFv-
IgAHWTG1C, which HAS the same sequence as the above construct. The polynucleotide
sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided
in SEQ ID NO:__.

EXAMPLE 107

20 L6 scFv VHL11S (SSS-S) H WCH2 WCH3

- This construct has a L6 single chain Fv with a mutation in the heavy chain
variable region at amino acid residue 11, where leucine has been changed to serine
according to the methods described in Example 49. This binding region is connected to a
mutated human IgG1 connecting region where all of the cysteines and one proline have been
25 changed to serines (SSS-S) according to methods described in Example 5. This connecting
region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in
Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded
polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 108

2H7 scFv-LLAMA IGG1

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a llama IgG1 hinge, CH2 and CH3 regions according to the methods described in Example 10. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 109

2H7 scFv-LLAMA IGG2

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a llama IgG2 hinge, CH2 and CH3 regions according to the methods described in Example 10. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 110

2H7 scFv-LLAMA IGG3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a llama IgG3 hinge, CH2 and CH3 regions according to the methods described in Example 10. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 111

CD16 LOW (ED)(SSS-S) H P238SCH2 WCH3

This construct has the extra cellular, CD16 low affinity allele binding domain as described in Example 43. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according

to methods described in Example 70. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 112

CD16-9 HIGH (ED)(SSS-S) H P238SCH2 WCH3

- 5 This construct has the extra cellular, CD16 high affinity allele binding domain as described in Example 43. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region.
- 10 The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

Example 113

2e12 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

- 15 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S
- 20 mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a human CD80 transmembrane and cytoplasmic tail region (hCD80 TM/CT). The hCD80 TM/CT was cloned using random primed cDNA derived from the BJAB cell line according to methods described in Example 12. This TM/CT region was attached to a Ig CH3 region with an
- 25 open reading frame (ORF). Open reading frame versions of scFvIg constructs of interest were created by replacement of the soluble versions of each –Ig tail with ORF (open reading frame versions) of these tails. PCR primers were designed for the existing clones of soluble –Ig tails which delete the stop codon and add one or more restriction sites to the 3' end of the new –Ig cassettes. The desired transmembrane and cytoplasmic tail sequences can then

connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. The polynucleotide sequence is provided in SEQ ID NO: ___, and the encoded polypeptide sequence is provided in SEQ ID NO: ___.

EXAMPLE 116

2H7 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S), according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. This construct has previously been referred to as: The polynucleotide sequence is provided in SEQ ID NO: ___, and the encoded polypeptide sequence is provided in SEQ ID NO: ___.

EXAMPLE 117

G19-4 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a G19 (anti-CD3) single chain Fv binding region described in Example 29. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT

2E12 scFv IgE CH2CH3CH4—hCD80TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. This CH4 region is attached to a hCD80 TM/CT essentially according to methods described in Example 113. The specific primers used to create an IgE ORF are listed below.

5' primer: 5'-gttggtgatcacgtctgctccaggacttcacc-3' (SEQ ID NO)

3'primer: 5'-gttggtttcgaaggatccgctttaccagattacagacaccgctcgctg-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 121

2E12 scFv (SSS-S)H P238SCH2 WCH3—mFADD-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a mouse FADD transmembrane and cytoplasmic tail region (mFADD TM/CT). This region is cloned using essentially the same methods described in Example 113. The domain was PCR amplified from randomly primed cDNA from mouse spleen RNA. The specific primers are listed below.

5' primer: 5'-gttggtgatccttcgaaccattcctggtgctgctgcactcgctg-3' (SEQ ID NO)

3' primer: 5'-gttggtatcgatctcgatcagggtgtttctgaggaagacacagt-3' (SEQ ID NO)

The specific primers used to create a mouse IgG ORF are listed below.

5'primer: 5'-gttgtagatctggagcccagagggcccacaatcaagccctctcctcaagcaaaagccca-3' (SEQ ID NO)

3'primer: 5'-gttggtttcgaaggatccgctttacccggagtcgggagaag-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 122

2E12 scFv (SSS-S)H WCH2 WCH3—mFADD-TM/CT

5 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The
10 CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 123

2E12 scFv (SSS-S)H WCH2 WCH3—mCASP3-TM/CT

15 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The
20 CH3 region is attached to a mouse casp3 transmembrane and cytoplasmic tail region according to methods described in Examples 113 and 121. The specific primers used to isolate the mcasp3 TM/CT region are listed below:

5' primer: 5'-gttgttgatccttcgaacatggagaacaacaaacctcagtgattca-3' (SEQ ID NO)

3' primer: 5'-gttgttatcgatctcgagctagtgtataaaagtacagttcttcgt-3' (SEQ ID NO)

25 The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 124

2E12 scFv (SSS-S)H P238SCH2 WCH3—mCASP3-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is connected to a mcasp3 TM/CT region according to methods described in Examples 113, 121 and 123. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 125

2E12 scFv (SSS-S)H WCH2 WCH3—MCASP8--TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a mouse casp8 transmembrane and cytoplasmic tail region (mcasp8 TM/CT) essentially according to methods described in Example 113 and 121. The specific primers used to clone the mcasp8 TM/CT region are listed below.

5' primer: 5'-gtgtttcgaacatggattccagagtgctcttatgctattgctg-3' (SEQ ID NO)

3' primer: 5'-gtgttatcgatctcgagtcattagggaggaagaagagcttctccg-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 126

2E12 scFv (SSS-S)H P238SCH2 WCH3—MCASP8-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines

(SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mcasp8 TM/CT region according to methods described in Examples 113, 121 and 125. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 127

2E12 scFv (SSS-S)H WCH2 WCH3—HCASP3--TM/CT

10 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The
15 CH3 region was attached to a human casp3 transmembrane and cytoplasmic tail region (hcasp3 TM/CT) essentially according to methods describe in Examples 113. The specific primers used to clone the hcasp3 TM/CT region are listed below.

5' Primer: 5'-gttgtggtccttcgaacatggagaacactgaaaactcagtggat-3' (SEQ ID NO)

3' Primer: 5'-gttgttatcgatctcgagttagtgataaaaatagagttctttgtgag-3' (SEQ ID NO)

20 The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 128

2E12 scFv (SSS-S)H P238SCH2 WCH3—HCASP3-TM/CT

25 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according

to methods described in Example 70. The CH3 region is attached to a hcasp3 TM/CT region according to methods described in Examples 113 and 127. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

5

EXAMPLE 129

2E12 scFv (SSS-S)H WCH2 WCH3—HCASP8--TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a human casp8 transmembrane and cytoplasmic tail region (hcasp8 TM/CT) according to methods described in Example 133. The specific primers used to clone the hcasp8 TM/CT region are listed below.

10 5' Primer: 5'-gttgtgcatccttcgaacatggacttcagcagaaatctttatgat-3' (SEQ ID NO)

3' Primer: 5'-gttgttatcgatgcagctcaatcagaagggaagacaagttttttct-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 130

20

2E12 scFv (SSS-S)H P238SCH2 WCH3—HCASP8-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129. The polynucleotide sequence is

25

provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 131

1D8 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

5 This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S
10 mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hCD80 TM/CT region according to methods described in Example 113. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

15 EXAMPLE 132

1D8 scFv (SSS-S)H WCH2 WCH3—hCD80TM/CT

 This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines
20 (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a hCD80 TM/CT region according to methods described in Example 113. This construct has previously been referred to as 1D8 scFv IgG WTH WTCH2CH3-CD80, which has the same sequence as the above construct. The
25 polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 133

1D8 scFv—mIgAH WIGA CH2 T4CH3—hCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 39. The CH3 region can be attached to a hCD80 TM/CT region according to methods described in Examples 113 using primers that create an IgA ORF.

EXAMPLE 134

1D8 scFv IgE CH2CH3CH4 -hCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. The CH4 region is attached to a hCD80 TM/CT according to methods described in Examples 113 and 120. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 135

1D8 scFv (SSS-S)H P238SCH2 WCH3—mFADD-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 136

1D8 scFv (SSS-S)H WCH2 WCH3—mFADD-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: ____.

EXAMPLE 137

1D8 scFv (SSS-S)H WCH2 WCH3—mcasp3-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region was attached to a mcasp3 TM/TM region according to methods described in Example 113, 121 and 123. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: ____.

EXAMPLE 138

1D8 scFv (SSS-S)H P238SCH2 WCH3—MCASP3-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mcasp3 TM/TM region according to methods described in Example 113, 121 and 123. The polynucleotide

sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 139

1D8 scFv (SSS-S)H WCH2 WCH3—MCASP8--TM/CT

5 This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The
10 CH3 region was attached to a mcasp8 TM/TM region according to methods described in Example 113, 121 and 125. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 140

1D8 scFv (SSS-S)H P238SCH2 WCH3—MCASP8-TM/CT

15 This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S
20 mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mcasp8 TM/TM region according to methods described in Example 113, 121 and 125. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

25

EXAMPLE 141

1D8 scFv (SSS-S)H WCH2 WCH3—HCASP3--TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1

connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a hcasp3 TM/CT according to methods described in Examples 113 and 127. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 142

1D8 scFv (SSS-S)H P238SCH2 WCH3—HCASP3-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp3 TM/CT according to methods described in Examples 113 and 127. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 143

1D8 scFv (SSS-S)H WCH2 WCH3—HCASP8--TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 144

1D8 scFv (SSS-s)H P238SCH2 WCH3—HCASP8-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 145

L6 scFv (SSS-S) H WCH2 WCH3

This construct has a L6 scFv binding domain as described in Example 105. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as L6 scFv-IgMHWGTG1C, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 146

2H7 scFv CD154 (L2)

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region has been attached to CD154 extracellular domain according to methods described in Example 4. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 147

2H7 scFv CD154 (S4)

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region has been attached to CD154 extracellular domain according to methods described in Example 4, such that methods of attachment resulted in a truncated version compared to the construct describe in Example 146. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 148

CTLA4 IgAH IgACH2CH3

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is attached to a wild type human IgA connecting region as described in Example 5. This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13. This constant region is attached to a J-chain region as described in Example 13. This construct has previously been referred to as CTLA-4 IgAH IgACH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 149

CTLA4 IgAH IgACH2 T4CH3

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This construct has previously been referred to as CTLA-4 IgAH IgA-T4, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 150

2H7 scFv IgAH IgACH2CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a wild type human IgA connecting region as described in Example 5. This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 151

2H7 scFv IgAH IgAHCH2 T18CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 18 amino acid residues prior to the 3' stop codon as described in Example 13. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 152

2H7-40.2.220 scFv (SSS-S) H WCH2 WCH3

A bispecific fusion protein was constructed between 2H7 scFv (anti-CD20) and 40.2.220 (anti-CD40), which both target B cell receptors. The 2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3 construct in the expression vector pD18 was passaged through dam⁻ bacteria in order to permit cleavage at the BclI site. Cleaved plasmid was treated with alkaline phosphatase prior to ligation to a BclI cut linker-CD40 scFv fragment. This fragment was synthesized from the existing 40.2.220 scFv by successive PCR reactions with overlapping primers. The linker attached is a previously patented (BMS patent issued) helical type linker with a high number of lysine and glutamic acid residues. The scFv for CD40 was PCR amplified without the leader peptide as a SalI-BclI fragment, but with the hinge type linker substituted at the amino terminus as a BclI-SalI fragment, to mediate insertion of the linker-scFv cassette as a BclI fragment between the scFv and -Ig tail

included in an existing scFvIg construct for CD20 (2H7 scFv hIgG1 constructs). The 3' end was similar to the other scFv VH molecules with an out of frame BclI site fused to the VTVSS type sequence at the end of the VH domain.

PCR oligos:

5 40.2.220 scFv:

5' primer--40.2.220S5: 5'-gttggtgacgacattgttctgactcagtcctccagccaccctgtc-3' (SEQ ID NO)

3' primer--40.2.220Bcl3: 5'-gttggtgatcagagacagtgtaccagtgctccttgg-3' (SEQ ID NO)

Linker Primers:

10 BclI-SalI fragment created by annealing complementary oligonucleotides. This fragment is then ligated into BclI digested vector along with a SalI-BclI scFv to create the (linker-scFv) BclI fragment desired for shuttling.

5' primer: 5'-gatcaatccaactctgaagaagcaaagaagaggaggccaaaaaggaggaagccaagaaatctaacagcg-3' (SEQ ID NO)

15 3' primer: 5'-tcgacgctgtagatttcttgcttcctccttttggcctcctcttcttcttcttcagagttggatt-3' (SEQ ID NO)

This BclI fragment was then ligated downstream of the 2H7 scFv in pD18-Ig. Transformants were screened for the presence of a 2.4 kb HindIII-Xba insert and positive clones sequenced prior to further studies. COS cell transient transfections were performed with this construct and culture supernatants screened for the presence of protein of the predicted size and for binding to CD20 and to CD40 transfected CHO cells.

20 New bispecific constructs can be created by designing hinge type linkers which incorporate one or preferably two restriction sites at either end of the linker, facilitating asymmetric digests and transfer of (linker-scFv) or (scFv-linker) cassettes between different constructs. These constructs will also incorporate the VH L11S and other V region substitutions, which presumably facilitate proper folding and result in increased expression of the molecules in which they are inserted.

25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been

referred to as anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

5

EXAMPLE 153

2H7 scFv IgAH IgACH2 T4CH3-hCD80 TM/CT

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113 and 119. This construct has previously been referred to as 2H7 scFv IgA hinge IgA-T4-CD80 and 2H7 scFv IgAH IgA-T4-CD80, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

10

15

EXAMPLE 154

G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD80 TM/CT

This construct has a G19 (anti-CD3) single chain Fv binding region described in Example 29. This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

20

25

EXAMPLE 155

2e12 scFv (CCC-P) WH WCH2 WCH3-hCD80 TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is attached to a wild type human IgG1

connecting region (CCC-P) as described in Example 1. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 156

2H7 VHL11S scFv (SSS-S) IgECH3CH4

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine. as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to human IgE CH3 and CH4 constant region. This truncated constant region was created according to the methods described in Example 38. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 157

IGD HINGE

An alternative hinge region can be isolated from human IgD immunoglobulin hinge region by using PCR assay to isolate the desired region. The PCR reaction is the same used in Example 1. This hinge was truncated by 6 amino acid residues at the 3' end. The primers used in this PCR reaction are listed below.

5'' Primer: 5'-GTGGATCCAGGTTCGAAGTCTCCAAAGGCACAGGCC-3' (SEQ ID NO)

3' primer: 5'-GTTGTCGACTGCACCGGTCTTTGTCTCTCTCTCTC-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 158

hCD28 TM/CT

For some of the cell surface ORF constructs, the transmembrane domain of CD80 was substituted with the transmembrane domain of human CD28 because it forms a dimer on the cell surface rather than a monomer as the CD80 does. Several of the molecules which drive the apoptotic program require oligomerization/trimerization to form a signaling complex; therefore, it is important to be able to control initiation of signaling by controlling the degree of oligomerization of these recombinant receptors on the cell surface.

The primers used in PCR amplification of the CD28 tail are given below:

5' Primer: 5'-gttgtggtccttcgaacccctttgggtgctggtggtggtggtgga-3' (SEQ ID NO)

10 3' primer: 5'-gttggtatcgatctcgagtcaggagcgataggctgcgaagtc-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 159

2H7 scFv VH L11S (SSS-S) H K322L CH2 WCH3

15 This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine. as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5.

20 The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322L mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a leucine using overlapping PCR described in Example 56, but with different primers for the first PCR reaction, which are listed below.

5' primer: 5'ttctcttcccccaaaacccaaggacacctcatgatctcccggaaccctgaggtcac-3' (SEQ ID NO)

25)

3' primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO)

PCR product was cloned into TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 160
2H7 scFv VH L11S (CSS-S) H K322L CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322L mutation in the CH2 region is at residue 322, where a Lysine has been changed to a leucine using overlapping PCR described in Example 56, using primers from Example 159 in the first PCR reaction and primers from Example 57 for the second PCR reaction.

PCR products were cloned into the TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

Additional representative constructs or sequences within various of examples of the present invention are as follows:

NT

HuIgG1 wild type hinge, CH2, CH3 (nucleotide sequence)

tctgatcaggagcccaaatctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagtcctc
ctctccccccaaaaccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaaga
ccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacag
cacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaag
ccctccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccc
gggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgaggag
caatgggcagccggagaacaactacaagaccacgcctcccggtgtggactccgacggctccttctctctacagcaagctcaccgt

ggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc
ctctccctgtctccggtaaataatgatctaga

AA

HuIgG1 wild type hinge, CH2, CH3 (amino acid sequence)

5 SDQEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK

10 NT

Llama IgG1 hinge, CH2, CH3 (nucleotide sequence)

tgatcaagaaccacatggaggatgcacgtgccncagtgccncaatgccnngcncngaactnccaggaggcccttctgtctttg
tcttcccccgaaaccaaggacgtcctctccatttttgaggccgagtcacgtgcgtttagtgagcgtcgaaagaaagaccccg
aggtcaattcaactggtatattgatggcgttgaggtgcgaacggccaatacgaagccaaaagaggaacagttcaacagcagctacc
15 gcgtggtcagcgtcctgcccattccagcaccaggactggctgacggggaaggaattcaagtgaaggtaacaacaaagctctccc
ggcccccatcgagaggaccatctccaaggccaaagggcagacccgggagccgcaggtgtacaccctggccccacaccgggaa
gaactggccaaggacaccgtgagcgtaacatgcctggtaaaaggcttctaccagctgacatcaacgttgagtggcagaggaacg
gtcagccggagtcagagggcacctacccaacacgccgccacagctggacaacgacgggacctaactctctacagcaagctctc
gggtggaaagaacacgtggcagcggggagaaaccttaacctgtgtggtgatgcatgaggccctgcacaaccactacaccagaaa
20 tccatcaccagctcttcgggtaaataatagtaatactaga

AA

Llama IgG1 hinge, CH2, CH3 (in figure 23 as Llama IgG1) (amino acid sequence)

EPHGGCTCPQCPAPELLPGGPSVFVFPPKPKDVLSISGRPEVTCVVVDVGKEDPEVNF
NWYIDGVEVRTANTKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPA
25 PIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRNGQP
ESEGTYANTPPQLDNDGTYFLYSRLSVGKNTWQRGETLTGVVMHEALHNHYTQKS
ITQSSGK

NT

Llama IgG2 (nucleotide sequence)

tgatcaagaaccaagacaccaaaccacaaccacaaccacaaccacaaccacaaccatcctacaacagaatccaagtgtcccaaatgtc
cagcccctgagctcctgggaggccctcagtcttcatcttccccgaaaccaaggacgtcctctccatttctgggaggcccagg
5 tcacgtgcgttggttagacgtgggccaggaagacccccgaggtcagttcaactggtacattgatggcgtgaggtgcgaacggcc
aacacgaggccaaaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgccatccagcaccaggactggctgacg
gggaaggaattcaagtgaaggtaacaacaagctctccggccccatcgagaagaccatctccaaggccaaaggcgagacc
cgggagccgcaggtgtacacctggccccacaccgggaagagctggccaaggacaccgtgagcgtaacatgcctggtcaaagg
cttctaccacctgatatcaacgttgaggcagaggaatgggcagccggagtcagagggcacytaccgccaccacgccacccag
10 ctggacaacgacgggacctacttctctacagcaagctctcgggtgggaaagaacacgtggcagcaggggaaaaccttcacctgtgt
ggtgatgcacgaggccctgcacaaccactacaccagaaatccatcaccagctctcgggtaaataagtaactaga

AA

Llama IgG2 (amino acid sequence)

DQEPKTPKPQPQPQPQNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLISGRPEVT
15 CVVVDVGQEDPEVSFNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWLT
GKEFKCKVNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKG
FYPPDINVEWQRNGQPESEGTYYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETFTC
VVMHEALHNHYTQKSITQSSGK

NT

20 **Llama IgG3 Fc (nucleotide sequence)**

tgatcaagcgcaccacagcgaagaccccagctccaagtgtcccaaatgccaggccctgaactccttgaggggcccacggcttca
tcttcccccgaaagccaaggacgtcctctccatcaccgaaaacctgaggtcacgtgcttggtggacgtgggtaaaagaagacc
tgagatcgagtcaagctggtccgtggatgacacagaggtacacacggctgagacaaagccaaaggaggaacagttcaacagcac
gtaccgcgtggtcagcgtcctgccatccagcaccaggactggctgacggggaaggaattcaagtgaaggtaacaacaagct
25 ctccagcccccatcgagaggaccttccaaggccaaagggcagaccgggagccgcaggtgtacacctggccccacaccg
ggaagagctggccaaggacaccgtgagcgtaacctgcctggtcaaaggcttctccagctgacatcaacgttgagtggcagagga
atgggcagccggagtcagagggcacctacgccaacacgccccacagctggacaacgacgggacctacttctctacagcaaac
tctcgtgggaaagaacacgtggcagcagggagaagtcttcacctgtgtggtgatgcacgaggctctacacaatcactccaccag
aaatccatcaccagctctcgggtaaataagtaactagagggcc

AA

Llama IgG3 Fc (amino acid sequence)

DQAHHSEDPSSKCPKCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKKT
LRSSSSWSVDDTEVHTAETKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNK
5 ALPAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVEWQR
NGQPESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHS
TQKSITQSSGK

NT

HuIgG1 wild type hinge (nucleotide sequence)

10 gatcaggagcccaaatcttgacaaaactcacacatgccaccgtgccagca

AA

HuIgG1 wild type hinge (amino acid sequence)

DQEPKSCDKTHTCPPCPA

NT

15 **HuIgG1 H2, wild type hinge with leu at second position (nucleotide sequence)**

gatctggagcccaaatcttgacaaaactcacacatgccaccgtgccagca

AA

HuIgG1 H2, wild type hinge with leu at second position (amino acid sequence)

DLEPKSCDKTHTCPPCPA

20 **NTHuIgG1 wild type CH2 (nucleotide sequence)**

cctgaactcctggggggaccgtcagtccttctctcccccaaaaccaaggacaccctcatgatctccggaccctgaggtcacat
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaa
ggagtacaagtgcaaggtctccaacaagccctcccagcccccatcgagaaaaccatctccaaagccaaa

25 AA

HuIgG1 wild type CH2(amino acid sequence)

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

NT

HuIgG1 wild type CH3 (nucleotide sequence)

5 gggcagccccgagaaccacaggtgtacaccctgcccccatcccgaggagatgaccaagaaccaggtcagcctgacctgcctg
gtcaaaggcttctatcccagcgacatgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccc
gtgctggactccgacggctccttctctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgct
ccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaata

AA

10 **HuIgG1 wild type CH3 (amino acid sequence)**

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSGDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

NT

HuIgG1 mutated hinge (C-C-C→S-S-S) (nucleotide sequence)

15 gatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagca

AA

HuIgG1 mutated hinge (C-C-C→S-S-S) (amino acid sequence)

DQEPKSSDKTHTSPSPA

NT

20 **HIgG1MTH WTCH2CH3 (mutant hinge with wild type CH2 and CH3 (reads from the hinge+Ig tail) (nucleotide sequence)**

tgatcaccceaaatcttctgacaaaactcacacatctccaccgtcctcagcacctgaactcctgggtggaccgtcagttcttcttccc
cccaaaaccaaggacacctcatgatctcccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgag
gtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaacagcacgtac
25 cgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccc
agcccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccggtatga
gctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgacatgccgtggagtgggagagcaatggg

cagccggagaacaactacaagaccacgcctcccgctggactccgacggctccttctctacagcaagctcaccgtggacaa
gagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccc
tgtctccgggtaaatgataatctaga

AA

5 **Mutant hinge, but wild type CH2 and CH3 (amino acid sequence)**

DHPKSSDKTHTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQ

10 KSLSLSPGK

NT

LLG1-5'bgl 35 mer Llama IgG1 5'

5'-gtt gtt gat caa gaa cca cat gga gga tgc acg tg-3'

NT

15 **LLG2-5'bgl 32 mer, Llama IgG2-5'**

5'-gtt gtt gat caa gaa ccc aag aca cca aaa cc-3'

NT

LLG3-5'bgl 33 mer, Llama IgG3-5'

5'-gtt gtt gat caa gcg cac cac agc gaa gac ccc-3'

20 NT

LLseqsense 19mer, llama sequencing primer

5'-ctg aga tcg agt tca gct g-3'

NT

LLseqAS 19 mer

25 5'-cct cct ttg gct ttg tct c-3'

NT

2H7 scFv llama IgG1 (nucleotide sequence)

aagcttgccgccatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagttacatgcactgg
taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
5 gtgggtctgggaccttctactctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
cccccacgttcgggtctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tcctacaatcagaagtcaaggggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac
10 atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaactcttactgggtacttcgatgtctggggcacaggggaccac
ggtcaccgtctcttctgatcaagaaccacatggaggatgcacgtgccncagtgccncaatgccngcncngaactnccagga
ggcccttctgtcttctgtctcccccgaaaccaaggacgtcctctccattttggaggccgagtcacgtgcgttgtagtgagcgtcgga
aagaaagaccccagggtcaatttcaactgggtatattgatggcgttgaggtgcgaacggccaatacgaagccaaaagaggaacagtt
caacagcacgtaccgcgtggtcagcgtcctgccccatccagcaccaggactggctgacggggaaggaaattcaagtgaagggtcaac
15 aacaaagctctcccgcccccatcgagaggaccatctccaaggccaaagggcagaccgggagccgcaggtgtacacctggc
cccacaccgggaagaactggccaaggacaccgtgagcgtaacatgcctgggtcaaaggcttctaccagctgacatcaacgttgagt
ggcagaggaacggtcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttctct
acagcaagctctcgggtgggaagaacacgtggcagcggggagaaaccttaacctgtgtggtgatgcatgaggccctgcacaacca
ctacaccagaaatccatcaccagctctcgggtaaatagtaattctaga

20 AA

2H7 scFv llama IgG1 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
25 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPHGGCTCPQCP
APELPGGPSVFVFPKPKDVLSIFGGRVTCVVVDVGKKDPEVNFNWDYIDGVEVRTA
NTKPKKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTR

EPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRNGQPESEGTYANTPPQL
DNDGTYFLYSKLSVGKNTWQRGETLTCVVMHEALHNHYTQKSITQSSGK

NT

2H7 scFv llama IgG2 (nucleotide sequence)

5 aagcttgccgcatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaattacatgcactgg
taccagcagaagccaggatcctccccaacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
gtgggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
cccccacgttcggtgctgggaccaagctggagctgaaagatggcgggtgctggcggtggtggatctggaggaggtgggagc
10 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctcggtctatttctgtgcaagagtgggtactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaagaacccaagacaccaaaccacaaccacaaccacaacccaatcctacaacagaatcca
15 agtgtcccaaatgtccagcccctgagctcctgggagggccctcagcttctcatcttcccccgaaacccaaggacgtcctctccatttct
gggaggccccgaggtcacgtgcgttggttagacgtgggccaggaagaccccgaggtcagtttcaactggtacattgatggcgtg
aggtgcgaacggccaacacgaggccaaaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgccccatccagcacc
aggactggctgacggggaaggaattcaagtcaaggtcaacaacaaagctctcccgcccccatcgagaagaccatctccaaggc
caaagggcagacccgggagccgaggtgtacacctggccccacaccgggaagagctggccaaggacaccgtgagcgtaacat
20 gcctggtcaaaggcttctacccacctgatatcaacgttgagtgagcagaggaatgggcagccggagtcagagggcacgtacgccac
cacgccacccagctggacaacgacgggacctacttctctacagcaagctctcggtgggaaagaacagctggcagcagggaga
aaccttcacctgtgtggtgatgcagaggccctgcacaaccactacaccagaaatccatcaccagctcttcgggtaaatagtaatct
aga

AA2H7 scFv llama IgG2 (amino acid sequence)

25 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKTPKPQPQPQP

QPNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLISGRPEVTCVVVDVGQEDPEVS
 FNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALP
 APIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPPDINVEWQRNG
 QPESEGTYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETFTCVVMHEALHNHYTQ
 5 KSITQSSGK

NT2H7 scFv llama IgG3 (nucleotide sequence)

aagcttgccgccatggattttcaagtcagattttcagcttcctgctaatacagtccttcagtcataattgccagaggacaaattgttctctc
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgaagttacatgcactgg
 taccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga
 10 gtgggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggtttaac
 ccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtgatctggaggaggtgggagc
 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
 15 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactgggtacttcgatgtctggggcacagggaccac
 ggtcaccgtctcttctgatcaagcgaaccacagcgaagaccccagctccaagtgcccaaatgccaggccctgaactccttgag
 ggcccacggtcttcatcttcccccgaaagccaaggacgtcctctccatcacccgaaaacctgaggtcacgtgcttgtggtggacgt
 gggtaagaagaccctgagatcgagttcaagctggctcgtggatgacacagaggtacacacggctgagacaaagccaaaggagg
 aacagttcaacagcacgtaccgcgtggtcagcgtcctgccccatccagcaccaggactggctgacggggaaggaattcaagtcaa
 20 ggtcaacaacaaagctctcccagcccccatcgagaggaccatctccaaggccaaagggcagacccgggagccgcaggtgtacac
 cctggccccacaccgggaagagctggccaaggacaccgtgagcgtaacctgcctggtcaaaggcttctcccagctgacatcaac
 gttgagtggcagaggaatgggcagccggagtcagaggcacctacgccaacacgccgccacagctggacaacgacgggaccta
 ctctctacagcaaactctcgtgggaaagaacacgtggcagcagggagaagtcttcacctgtgtggtgatgcacgagggtctaca
 caatcactccaccagaaatccatcacccagctcttcgggtaaatagtaattctagagggcc

25 AA

2H7 scFv llama IgG3 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
 QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG

YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
 QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQAHSHSEDPSSKCP
 KCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKKTLRSSSSWSVDDTEV
 HTAETKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPIERTISKAKG
 5 QTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVEWQRNGQPESEGTYANTP
 PQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHSTQKSITQSSGK

NT

2H7+Completely WT IgG tail (nucleotide sequence)

2H7 scFv WTH WTCH2CH3 (nucleotide sequence)

10 aagcttgccgcatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcaggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga
 gtgggtctgggaccttactcttcacaatcagcagagtggaggctgaagatgtgccacttattactgccagcagtgaggtttaac
 ccacccacgttcggtgctgggaccaagctggagctgaagatggcgggtggctcggcggtggtgatctggaggaggtgggagc
 15 tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtgatact
 tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
 atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttactggtacttcgatgtctggggcacagggaccac
 ggtcaccgtctcttctgatcaggagcccaatctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggg
 20 accgtcagttcttcttcccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtg
 agccacgaagaccctgaggtcaagtcaactgttacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggag
 cagtacaacagcacgtaccgtgtggtcagcgtctcaccgtctgcaccaggactgggtgaatggcaaggagtacaagtgaaggt
 ctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacct
 gccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtgg
 25 agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagc
 aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
 gcagaagagcctctccctgtctccgggtaaatgatctaga

AA

2H7 scFv WTH WTCH2CH3 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
 QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
 PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
 5 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDKTHTCPP
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPP
 VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

10 **NTCD80 transmembrane domain and cytoplasmic tail (+restriction sites) (nucleotide sequence)**

gcggatccttgaacctgctcccatcctgggccattaccttaatctcagtaaagggaattttgtgatatgctgcctgacctactgctttgc
 cccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgat

AA

15 **CD80 transmembrane domain and cytoplasmic tail (amino acid sequence)**

ADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

40.2.220 VL (anti-human CD40 scFv #1--VL) (nucleotide sequence)

aagcttatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgttctgactca
 20 gtctccagccaccctgtctgtgactccaggagatagagtctctcttctcctgcagggccagccagagtattagcgactacttacactgg
 atcaacaaaaatcacatgagtctccaaggcttctcatcaaatatgcttccattccatctctgggatcccctccagggtcagtggcagtg
 gatcagggtcagatttcactctcagtatcaacagtggtgaacctgaagatgttggaatttattactgtcaacatgggtcacagctttccgtg
 gacgttcggtggaggcaccaagctggaaatcaaacgg

AA

25 **40.2.220 VL (anti-human CD40 scFv #1--VL) (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSFP
WTFGGGTKLEIKR

NT

5 **40.2.220 VH (for anti-human CD40 scFv #1--VH) (nucleotide sequence)**

cagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggctctgggtatgccttca
caactactggaatgcagtggtgcaagagatgccaggaaagggttgagtggattggctggataaacaccccactctggagtgcc
aaaatatgtagaagacttcaaggacggttgccttctcttggaaacctctgccaacactgcataattacagataagcaacctcaaagat
gaggacacggctacgtattctgtgtgagatccgggaatgtaactatgacctggcctactttgcttactggggccaaggacactgg
10 tctactgtctgatca

AA

40.2.220 VH (for anti-human CD40 scFv #1--VH) (amino acid sequence)

QIQLVQSGPELKKPGETVRISCKASGYAFTTTGMQWVQEMPGKGLKWIGWINTPL
WSAKICRRLQGRFAFSLETSANTAYLQISNLKDEDTATYFCVRSNGNGNYDLAYFAY
15 WGQGTLLTVS

NT

40.2.220 scFv (anti-human CD40 scFv #1) (nucleotide sequence)

aagcttatggatttcaagtcagatttctcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgttctgactca
gtctccagccaccctgtctgtgactccaggagatagagtctcttctcctgcagggccagccagagtattagcgactacttacactggt
20 atcaacaaaaatcacatgagttccaaggcttctcatcaaatatgcttccattccatctctgggatcccctccaggttcagtggcagtg
gatcagggtcagatttactctcagtatcaacagtggtgaacctgaagatgttggaatttactgtcaacatggtcacagcttccgtg
gacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcggcgagggtgggtcgggtggcggcggatctc
agatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtatgccttcac
aactactggaatgcagtggtgcaagagatgccaggaaagggttgagtggattggctggataaacaccccactctggagtcca
25 aaatatgtagaagacttcaaggacggttgccttctcttggaaacctctgccaacactgcataattacagataagcaacctcaaagatg
aggacacggctacgtattctgtgtgagatccgggaatgtaactatgacctggcctactttgcttactggggccaaggacactggt
cactgtctgatca

AA

40.2.220 scFv (anti-human CD40 scFv #1) (amino acid sequence)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSFP
5 WFTGGGTTKLEIKRGGGGSGGGGSGGGGSGGIQLVQSGPELKKPGETVRISCKASGYA
FTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAYLQIS
NLKDEDTATYFCVRSNGNGNYDLAYFAYWGQGTLVTVS

NT

2e12 VL (with L6 VK leader peptide) (nucleotide sequence)

10 atggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgctcacccaatctcc
agcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaggtgtgaatattatgtcacaagttaatgcag
tggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctgggggtccctgccaggttagtg
gcagtggtgctgggacagacttcagcctcaacatccatcctgtggaggaggatgatatgcaatgtatttctgtcagcaaagtaggaa
gggtccttgacgttcggtggaggcaccaagctggaaatcaaacgg

15 AA

2e12 VL (with L6 VK leader peptide) (amino acid sequence)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL
MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ
QSRKVPWFTFGGGTKLEIKR

20 NT

2e12 VH (no leader peptide) (nucleotide sequence)

caggtgcagctgaaggagtcaggacctggcctggcggcgcctcacagagcctgtccatcacatgcaccgtctcagggttctcatta
accggctatgggtgtaaaactgggttcgccagcctccaggaaagggtctggagtggtgggaatgatatgggggtgatggaagcacag
actataattcagctctcaaataccagactgagcatcaccaaggacaactccaagagccaagttttctaaaaatgaacagctctgcaaact
25 gatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactggggtcaaggaacctcagtc
accgtctcctca(gatctg)

AA

2e12 VH (amino acid sequence)

QVQLKESGPGLVAPSQSL SITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDGS
TDYNSALKSRLSITKDNSKSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMDY
WGQGTSVTVSS

5 NT

2e12scFv(+Restriction sites) (nucleotide sequence)

aagcttatggatttcaagtcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaaagtggtgaatattatgtcacaaagtta
atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgtgcatccaacgtagaatctggggtcctgccagg
10 tttagtggcagtggtctgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtattctgtcagcaaagt
aggaaggttccttgacgttcggtggagggcaccaagctggaaatcaaacggggtggcgggtggctcgggagggtgggtcgggt
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggtgggaatgatatgggggtg
atggaagcacagactataattcagctctcaaataccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaaatgaa
15 cagctctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa
ggaacctcagtcaccgtctcctct(gatcag)

AA

2e12scFv (amino acid sequence)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL
20 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ
QSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSL SITC
TVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDGSTDYNSALKSRLSITKDNSKSQ
VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQGTSVTVSS

NT

25 **10A8 is anti-CD152 (CTLA-4)**

10A8 VL (with L6 VK leader peptide) (nucleotide sequence)

atggatttcaagtcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacacagctctc
catcctcactgtctgcatctctgggaggcaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttggtacca

acacaagcctggaaaagggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtggaaagtggg
tctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataatcttcattgac
gttcggctcggggacaaagttggaataaaacgg

AA

5 **10A8 VL (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIQMTQSPSSLSASLGKVTITCKASQDIKKYIGW
YQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSRDYSLSIRNLEPEDIATYYCQQYDN
LPLTFGSGTKLEIKR

NT

10 **10A8 VH (no leader peptide) (nucleotide sequence)**

gatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtcctctgtctctcacctgctctgtcactggctactccatcacc
agtggtttctactggaactggatccgacagttccgggaaacaaactggaatggatgggccacataagccacgacggtaggaataac
tacaacctctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtgactactgagga
cacagctacatatttctgtgcaagacactacggtagtagcggagctatggactactgggggtcaaggaacctcagtcaccgtctcctct
gatca

AA

10A8 VH (amino acid sequence)

DVQLQESGPGLVKPSQSLTCSVTGYSITSGFYWNWIRQFPGNKLEWMGHISHDG
RNNYNPSLINRISITRDTSKNQFFLKLSSVTTEDTATYFCARHYGSSGAMDYWGQGT
SVTVSS

NT

10A8 SCFV (nucleotide sequence)

aagcttatggattttcaagtcagattttcagttcctgctaatacagtcgttcagtcataatgtccagaggagtcgacatccagatgacac
agtcctcatcctcactgtctgcatctctgggaggcaaaagtcacatcacttgcaaggcaagccaagacattaagaagtatataggttg
taccaacacaagcctggaaaagggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtggaa
gtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataatcttcc
attgacgttcggctcggggacaaagttggaataaaacgggggtggcgggtggctcgggcggtgggtgggtcgggtggcggcggtatct

gatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtcctctgtctctcacctgctctgtcactggctactccatcacc
agtggtttctactggaactggatccgacagtttccgggaacaaactggaatggatgggccacataagccacgacggtaggaataac
tacaacctatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtgactactgagga
cacagctacatatttctgtgcaagacactacggtagtagcggagctatggactactggggtaaggaacctcagtcaccgtctcctct
5 gatca

AA

10A8 SCFV (amino acid sequence)

MDFQVQIFSFLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIGW
YQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSRDYSLIRNLEPEDIATYYCQQYDN
10 LPLTFGSGTKLEIKRGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSVTGY
SITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFLKLSS
VTTEDTATYFCARHYGSSGAMDYWGQGTSTVTVSSD

NT

40.2.220-hm1IgG1-hCD80 (nucleotide sequence)

15 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgttctgactca
gtctccagccaccctgtctgtgactccaggagatagagtctctcttctgcagggccagccagagtattagcgactacttacactggt
atcaacaaaaatcacatgagtcctcaaggcttctcatcaaatatgcttccattccatctctgggatcccctccagggtcagtggcagtg
gatcagggtcagatttactctcagtatcaacagtggtgaacctgaagatgttggaatttattactgtcaacatggtcacagctttccgtg
gacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggtggcggcggatctc
20 agatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtatgccttcac
aactactggaatgcagtggtgcaagagatgccaggaaagggttggaaggattggctggataaacacccactctggagtgcca
aaatatgtagaagacttcaaggacggttgccttctcttggaaacctctgccaacactgcataattacagataagcaacctcaaagatg
aggacacggctacgtatttctgtgtgagatccgggaatggttaactatgacctggcctactttgcttactggggccaagggaactggt
cactgtctctgatctggagcccaaatctctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatcgtca
25 gtcttctcttcccccaaaaccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccac
gaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtac
aacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtagaagtgcaaggtctccaa
caaagccctcccagccccatcgagaaaacatctcaaagccaaagggcagccccgagaaccacaggtgtacacctgcccc
atcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtggg

tggggggatcgtcagttcttcttcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtgggtggt
 ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
 ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaag
 tgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt
 5 acaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc
 gccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
 cactacacgcagaagagcctctccctgtctccgggtaaagcggtaccttccaacctgctcccatcctgggccattacctaatactcagt
 aaatggaattttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaaggga
 10 aagtgtacgccctgtataaatcgat

AA

2e12scFv- hmtIgG1-CD80 fusion protein (amino acid sequence)

MDFVQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL
 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ
 15 QSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLITC
 TVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNSKSQ
 VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWQGTSVTVSDDLPEKSSDKT
 HTSPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 20 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFNCSVMHEALHNHYTQKSLSLSPGKA
 DPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

10A8 scFv-hmtIgG1-CD80 (nucleotide sequence)

25 aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacac
 agtctccatcctcactgtctgcatctctgggaggcaaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttgg
 taccaacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtgga
 gtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataatcttc
 attgacgttcggctcggggacaaagttggaaataaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatc

gatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtctctgtctctcacctgctctgtcactggctactccatcacc
 agtggtttctactggaactggatccgacagttccgggaacaaactggaatggatgggccacataagccacgacggtaggaataac
 tacaacctatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttctgaagttgagttctgtgactactgagga
 cacagctacataatttctgtgcaagacactacggtagtagcggagctatggactactggggtaaggaacctcagtcaccgtctcctct
 5 gatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatcgtcagttctcctct
 cccccaaaaccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtgggtgggacgtgagccacgaagaccct
 gaggtcaagttcaactggtagctggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
 taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccct
 ccagcccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccggga
 10 tgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtggagtgaggagcaatg
 ggcagccggagaacaactacaagaccacgcctcccgctgtgactccgacggctccttctcctctacagcaagctcaccgtggac
 aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc
 cctgtctccgggtaaagcggatccttgaacctgtcccatcctggggccattaccttaatctcagtaaatggaattttgtgatatgtgc
 ctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcga
 15 t

AA

10A8 scFv-hmIgG1-CD80 (amino acid sequence)

MDFQVQIFSLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIGW
 YQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGGRDYSLSIRNLEPEDIATYYCQQYDN
 20 LPLTFGSGTKLEIKRGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSVTGY
 SITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFLKLSS
 VTTEDTATYFCARHYGSSGAMDYWGQGTSTVTVSSDLEPKSSDKTHTSPSPAPELL
 GGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 25 VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSF
 FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPSWAIT
 LISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

500A2-hmtIgG1-CD80 (nucleotide sequence)

atgttgatacatctcagctccttgggcttttactcttctggatttcagcctccagaagtacatagtctgactcagactccagccactct
gtctctaattcctggagaaagagtcacaatgacctgtaagaccagtcagaatattggcacaattctacactgggtatcaccaaaaaccaa
aggaggctccaagggctctcatcaagtatgcttcgcagtccattcctgggatccctccagattcagtggcagtggttcggaacaga
5 tttactctcagcatcaataacctggagcctgatgatatcggaatttattactgtcaacaaagtagaagctggcctgtcacgttcggtcct
ggcaccaagctggagataaaacggggtggcgggtggctcgggcggaggtgggtcgggtggcggcggtatcaggtcaagctgca
gcagtcgggttctgaactagggaacctggggcctcagtgaactgtcctgcaagacttcaggctacatattcacagatcactatatt
cttgggtgaaacagaagcctggagaaagcctgcagtgataggaaatgtttatggtggaaatggtggtacaagctacaatcaaaaatt
ccagggcaaggccacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgaggattctgccat
10 ctattactgtgcaagaaggccggtagcgacgggcatgctatggactactggggtcaggggatccaagttaccgtctcctctgatctg
gagcccaaatcttctgacaaaactcacacatccccaccgtcccagcacctgaactcctggggggatcgtcagttctctcttcccc
caaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggt
caagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaaagccgcgggaggagcagtacaacagcacgtaccg
tgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggttccaacaaagccctccag
15 ccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggtatgagc
tgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggca
gccggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttctcctctacagcaagctcaccgtggacaaga
gcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgcagaagagcctctccctgt
ctccgggtaaagcggatccttgaacctgtcccatcctgggccattacctaatactcagtaaatggaattttgtgatgtctgcctgac
20 ctactgctttgccccagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgcctgtataaatcgat

AA

500A2-hmtIgG1-CD80 (amino acid sequence)

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYHQ
KPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPVTF
25 GPGTKLEIKRGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIFTD
HYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMELSSLT
SEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSDLEPKSSDKTHTSPSPAPELLGG
SSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY

TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKADPSNLLPSWAITLI
SVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

5 **2H7 scFv MTH(SSS)WTCH2CH3 (nucleotide sequence)**

aagcttgccgcatggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcaaggccagctcaagtgaagttacatgcactgg
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca
gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
10 ccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagctggtagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtatact
tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttctactgggtacttcgatgtctggggcacagggaccac
15 gggtaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcccagcacctgaactcctgggggga
ccgtcagctcttcttctccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgggtggacgtga
gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggctc
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctg
20 ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttctctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgcatgaggctctgcacaaccactacag
cagaagagcctctccctgtctccgggtaaatgatctaga

AA

25 **2H7 scFv MTH(SSS)WTCH2CH3 (amino acid sequence)**

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM

QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
5 VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

NT

HuIgGMHncs1 (oligo for CSS) (nucleotide sequence)

ggt ggt gat cag gag ccc aaa tct tgt gac aaa act cac

NT

10 **HuIgGMHncs2 (oligo for SCS=ncs2) (nucleotide sequence)**

ggt ggt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg

NT

HuIgGMHncs3 (oligo for SSC=ncs3) (nucleotide sequence)

ggt ggt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca gca cct g

15 NT

hIgGWT3xba (3' oligo for above mutation introduction) (nucleotide sequence)

ggt ggt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g

NT

Vhser11: (oligo for Leu to Ser at VH11) (nucleotide sequence)

20 gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc

NT

huIgG1-3' (3' oligo to amplify IgG1 C regions, 3' end of CH3) (nucleotide sequence)

gtc tct aga cta tca ttt acc cgg aga cag

NT

25 **huIgA/Gchim5 (oligo for pcr#1) (nucleotide sequence)**

cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg

NT

huIgAhg-5' (oligo for pcr#2) (nucleotide sequence)

ggt ggt gat cag cca ggt ccc tca act cca cct acc cca tct ccc caa ct

NT

5 **huIgA3' (nucleotide sequence)**

ggt ggt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac

NT

2H7 scFv IgAH IGG WT CH2CH3 (2H7 scFv with IgA hinge and WT CH2 and CH3)
(nucleotide sequence)

10 aagcttgccgcatggattttcaagtgcagattttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcatctccaggggagaaggcacaatgacttcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
gtgggtctgggacctcttactcttcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggttttaac
cccccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
15 tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtatact
tctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaactcttactgggtacttcgatgtctggggcacagggaccac
ggtcaccgtctctgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgcgcacctga
20 actcctggggggaccgtcagttctcttccccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgt
gggtgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagt
acaagtgaaggtctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccac
aggtgtacacctgcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatccagc
25 gacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctcc
ttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtttctcatgctccgtgatgcatgaggctctgc
acaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

AA

2H7 scFv IgAH IGG WT CH2CH3 (amino acid sequence)

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
5 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSDQVPSTPPTPSPSTPP
TPSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
10 TPPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

NT

2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgAhinge and IgA CH2 and CH3) (nucleotide sequence)

aagcttgccgccatggatttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
15 ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgaagtacatgcactgg
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga
gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
cccccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtgatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat
20 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtgatact
tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgtgccac
ccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag
25 agatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctg
ctacagcgtgtccagtgctctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgtgcctaccccgagtc
caagaccccgtaccgccacctctcaaaatccggaaacacattccggcccaggtccacctgctgccgccgctcgaggag
ctggccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagccccaggatgtgctgggtcgctgggtgcaggggctc
acaggagctgccccgcgagaagtacgtgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgtgtgaccag

catactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgtggccttc
acacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgttgcacggcgaggtggacggcacctgctac
tgataatctaga

AA

5 **2H7 scFv IgAH IgACH2CH3** (2H7 scFv IgA hinge and IgA CH2 and CH3) (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
10 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQVPSTPPTPSPSTP
PTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWTPSSGKSAVQG
PPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEV
HLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQ
15 GTTTFVAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSV
VMAEVDGTCY

NT

IgA hinge-CH2-CH3 (Human IgA tail, full length) (nucleotide sequence)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcact
20 gcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg
tcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtcca
gtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgctgcctaccccgagtccaagacccccgcta
accgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccgtcgaggagctggccctgaacg
agctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccc
25 cgcgagaagtacgtgacttgggcatcccgaggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgtgg
cagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgtggccttcacacagaagacca
tcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgttgcacggcgaggtggacggcacctgctactgataatctaga

AA

IgA hinge-CH2-CH3 (Human IgA tail, full length) (amino acid sequence)

DQVPVSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVT
FTWTPSSGKSAVQGGPPDRDLGCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP
5 REKYLTWASRQEPSQGTTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKT
IDRLAGKPTHVNVSVVMAEVDGTCY

NT

Human J Chain (nucleotide sequence)

10 agatctcaagaagatgaaaggattgttcttgttgacaacaaatgtaagtgtgcccgattacttcaggatcatccgttctccgaagatc
ctaagaggacattgtggagagaaacatccgaattattgtcctctgaacaacaggaggagaatatctctgatccacctcaccattgaga
accagatttgtgtaccatttgtctgacctcagctgtaaaaatgtatcctacagaagtggagctggataatcagatagttactgctacc
agagcaatatctgtgatgaagacagtgtacagagacctgtacacttatgacagaaacaagtgtacacagctgtgggtccactcgt
atatggtggtgagacaaaatggtggaaacagccttaacccagatgcctgctatcctgactaatctaga

15 AA

Human J Chain (amino acid sequence)

RSQEDERIVLVDNKCKCARITSRIIRSSDPNEDIVERNIRIIVPLNNRENISDPTSPLRT
RFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVV
PLVYGGETKMOVETALTPDACYP

20 NT

HUJCH5nl (J chain 5' primer) (nucleotide sequence)

ggt ggt aga tct caa gaa gat gaa agg att ggt ctt

NT

HUJCH3 (J chain 3' primer-antisense) (nucleotide sequence)

25 ggt ggt tct aga tta gtc agg ata gca ggc atc tgg

AA

4 carboxy terminal amino acids deleted from IgA CH3 (amino acid sequence)

GTCY

NT

IgAH IgAT4 (human IgA tail, truncated (3T1)-(missing last 4 amino acids from carboxy terminus) (nucleotide sequence)

5 tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcact
gcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg
tcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgtacagcgtgtcca
gtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcactgctgcctaccccgagtccaagacccccgcta
accgccaccctctcaaaatccggaacacattccggcccgaggtccacctgctgccgccgccgtcgaggagctggccctgaacg
10 agctggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccc
cgcgagaagtacgtgacttgggcatcccgagcagggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgtgg
cagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcacacagaagacca
tcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgtgtcatggcggaggtggactgataatctaga

AA

15 **IgAH IgAT4** (amino acid sequence)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVT
FTWTPSSGKSAVQGPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP
REKYLTWASRQEPSQGTTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKT
20 IDRLAGKPTHVNVSVVMAEVD

NT

HUIGA3T1 (oligo 3' to delete 4 amino acids at carboxy end of IgA CH3)

ggt gtt tct aga tta tca gtc cac ctc cgc cat gac aac aga cac

NT

25 **HUIGA3T2** (oligo to delete 14 aa at end of IgA -T4)

ggt gtt tct aga tta tca ttt acc cgc caa gcg gtc gat ggt ctt

NT

2H7 scFv IgAH IgAT4 (2H7 scFv IgA 3T1 construct; truncates the CH3 domain at the 3'end) (nucleotide sequence)

aagcttgccgccatggattttcaagtcagatttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgaagttacatgcactgg
5 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctgcttcagtggca
gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
10 tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttacttggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccac
ccccgactgtcactgcaccgaccggccctcaggacactgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag
agatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctg
15 ctacagcgtgtccagtgctctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgctgcttacctacccgagtc
caagaccccgctaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccgtcggaggag
ctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctgggtcgtggctgcaggggtc
acaggagctgccccgcgagaagtacgtgacttgggcacatccggcaggagcccagggcaccaccaccttcgctgtgaccag
catactgcgcgtggcagccgaggactggaagaagggggacacctctcctgcatggtgggccacgaggccctgccgctggccttc
20 acacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcacatggcggaggtggactgataatctaga

AA

2H7 scFv IgAH-T4 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSPGEEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
25 PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWFVDVWGTGTTVTVSSDQVPSTPPTPSPSTP
PTPSPSCCHPRLSLHRPALEDLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQG
PPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEV

HLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQ
GTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSV
VMAEVD

AA

- 5 **14 amino acids deleted from IgAH-T4** (so that total of 18 amino acids deleted from wild type IgA CH3) (amino acid sequence)

PTHVNVSVVMAEVD

NT

IgAH IgA-T18 (human IgA Tail truncated, 3T2) (nucleotide sequence)

- 10 tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcact
gcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg
tcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtcca
gtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgtgcctaccccgagtccaagacccccgcta
accgccaccctctcaaaatccggaacacattccggcccgaggccacctgctgccgcccgctcgaggagctggccctgaacg
15 agctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccc
cgcgagaagtacgtgactgggcattccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcactgctgcgctgg
cagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcacacagaagacca
tcgaccgcttggcgggtaaa

AA

- 20 **IgAH IgA-T18** (amino acid sequence)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVT
FTWTPSSGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP
REKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKT

- 25 IDRLAGK

NT

2H7 scFv IgAH IgAT18 (human IgA Tail truncated, 3T2) (nucleotide sequence)

aagcttgccgcatggattttcaagtgcagattttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgaggccagctcaagtgttaattacatgcactgg
 taccagcagaagccaggatcctcccccaccctggatttatgcccataccctggcttctggagtcctgctcgcttcagtgga
 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 5 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
 tctcaggcttatctacagcagctctggggctgagctggtaggacctggggcctcagtgaaagatgctcgaaggcttctggctacacat
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
 atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaccac
 10 ggtaaccgtctcttctgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgctgccac
 ccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag
 agatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgtcaaggaccacctgaccgtgacctctgtggctg
 ctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgtgcttaccgagtc
 caagaccccgctaaccgccacctctcaaaatccggaaacacattccggcccaggtccacctgctgccgccgctcggaggag
 15 ctggccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtc
 acaggagctgccccgcgagaagtacctgactgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccag
 catactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggggccacgaggccctgccgctggccttc
 acacagaagaccatcgaccgcttggcgggtaaa

AA

20 **2H7 scFv IgAH IgAT18 (amino acid sequence)**

MDFVQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
 QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
 PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
 25 QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQVPSTPPTPSPSTP
 PTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWTPSSGKSAVQG
 PPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEV
 HLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQ
 GTTTFVAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGK

NT

CTLA-4 IgG WTH WTCH2CH3 (human-oncoMLP-CTLA4EC-hIgGWT) (nucleotide sequence)

gcaacctacatgatggggaatgagttgaccttcctagatgattccatctgcacgggcacctccagtggaatcaagtgaacctactat
5 ccaaggactgagggccatggacacgggactctacatctgcaaggtggagctcatgtaccaccgccatactacctgggcataggca
acggaaccagatttatgtaattgatccagaaccgtgcccagattctgatcaacccaaatctgtgacaaaactcacacatgccaccg
tgcccagcacctgaactcctggggggaccgtcagttctctcttcccccaaaacccaaggacacctcatgatctccggaccct
gaggtcacatgcgtggtgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcata
atgccaagacaaagccgcgaggagcagtacaacagcacgtaccgtgtgtcagcgtctcaccgtcctgcaccaggactggct
10 gaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaaggga
gccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaa
ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgtg
gactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgttctctcatgctccgtg
atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

15 AA

CTLA-4 IgG WTH WTCH2CH3 (amino acid sequence)

MGVLLTQRTL LSLVLALLFPSMASMAMHVAQPAVV LASSRGIASFVCEYASPGKAT
EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM
DTGLYICKVELMYPPPYL LGIGNGTQIYVIDPEPCPDSDQPKSCDKTHTCPPCPAPEL
20 LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

25 **Human OncoM leader Peptide+CTLA4 EC (BclII) (nucleotide sequence)**

atgggggtactgctcacacagaggacgtgctcagctgtgctccttgactcctgtttccaaagcatggcgagcatggcaatgcacgtg
gccagcctgctgtgtgactggccagcagccgaggcatgccagctttgtgtgtgagtatgcattccaggcaaagccactgaggtc
cgggtgacagtgtctcggcaggctgacagccaggtgactgaagctgtgctgcaacctacatgatggggaatgagttgaccttcta
gatgattccatctgcacgggcacctccagtggaatcaagtgaacctactatccaaggactgagggccatggacacgggactctac

atctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatccagaaccgt
gccagattctgatcaa

AA

Human OncoM leader Peptide+CTLA4 EC (amino acid sequence)

5 MGVLLTQRTLTLVLALLFPSMASMAMHVAQPAVVLASSRGIA SFVCEYASPGKAT
EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM
DTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDSQ

NT

Human OncoM leader (nucleotide sequence)

10 atgggggtactgctcacacagaggacgctgctcagctctggctcctgcactcctgtttccaagcatggcgagcatg

AA

Human OncoM leader (amino acid sequence)

MGVLLTQRTLTLVLALLFPSM

NT

15 **Human CTLA4 EC (no LP) (nucleotide sequence)**

gcaatgcacgtggcccagcctgctgtgtgtactggccagcagccgaggcatgccagcttgtgtgtgagtatgcatctccaggcaaa
gccactgaggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtcggcaacctacatgacggggaatg
agttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgaggccatgga
cacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaaccagatttatgtaatt
20 gatccagaaccgtgccagattct

AA

Human CTLA4 EC (no LP) (amino acid sequence)

AMHVAQPAVVLASSRGIA SFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMT
GNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLIGINGTQ
25 IYVIDPEPCPDS

NT

Human CTLA4 IgG MTH (SSS) MTCH2CH3 (nucleotide sequence)

atgggggtactgctcacacagaggacgctgctcagctgtgtccttgcaactcctgttccaagcatggcgagcatggcaatgcacgtg
gcccagcctgctgtggtactggccagcagccgagggcatgccagctttgtgtgtgagtatgcacatccaggcaaagccactgaggtc
cgggtgacagtgttcggcaggtgacagccaggtgactgaagtctgtcggcaacctacatgatggggaatgagttgaccttcta
5 gatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactatccaaggactgagggccatggacacgggactctac
atctgcaaggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatccagaaccgt
gcccagattctgatcaacccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatcgtcagt
cttctcttccccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtgagccacga
agacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagcagtagaa
10 cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtctccaaca
aagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctgccccat
ccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtggagtggga
gagcaatgggcagccgggagaacaactacaagaccacgcctccgtgctggactccgacggctcttctctctacagaagctca
ccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcataaggctctgcacaaccactacacgcagaa
15 gagcctctccctgtctccgggtaaatga

AA

Human CTLA4 IgG MTH (SSS) MTCH2CH3 (amino acid sequence)

MGVLLTQRTL LSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKAT
EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM
20 DTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDSQPKSSDKTHTSPSPAPELL
GGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK

25 NT

CTLA-4 IgAH IgACH2CH3 (human-oncoMLP-CTLA4EC-IgA) (nucleotide sequence)

atgggggtactgctcacacagaggacgctgctcagctgtgtccttgcaactcctgttccaagcatggcgagcatggcaatgcacgtg
gcccagcctgctgtggtactggccagcagccgagggcatgccagctttgtgtgtgagtatgcacatccaggcaaagccactgaggtc
cgggtgacagtgttcggcaggtgacagccaggtgactgaagtctgtcggcaacctacatgatggggaatgagttgaccttcta

gatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactatccaaggactgagggccatggacacgggactctac
 atctgcaaggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatccagaaccgt
 gccagattctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccaccccc
 gactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagat
 5 gacctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctgacctctgtggctgttac
 agcgtgtccagtgtctgcccgggtgtgccgagccatggaaccatgggaagaccttcacttgactgtgcctaccccagtgccaag
 accccgctaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccgtcggaggagctgg
 ccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacag
 gagtgccccgcgagaagtacctgactgggcatcccgccaggagcccagggcaccaccaccttcgctgtgaccagcata
 10 ctgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgtggccttcaca
 cagaagaccatcgaccgcttgccgggtaaacccacccatgtcaatgtgtctgttgcacggcgagggtggacggcacctgctactga
 taatctaga

AA

CTLA-4 IgAH IgACH2CH3 (amino acid sequence)

15 MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIA SFVCEYASPGKAT
 EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM
 DTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDSDQVPSTPPTPSPSTPPTSPS
 CCHPRLSLHRPALEDLLL GSEAILTCTLTGLRDASGVFTFTWTPSSGKSAVQGPPDRD
 LCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPP
 20 PSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTTF
 AVTSILRVA AEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAE
 VDGTCY

NT

CTLA-4 IgAH IgA-T4 (human-oncoMLP-CTLA4EC-IgA3T1) (nucleotide sequence)

25 atgggggtactgtcacacagaggacgtgctcagctgtgctcctgcactcctgtttccaagcatggcgagcatggcaatgcacgtg
 gccagcctgctgtggtactggccagcagccgaggcatcgccagcttctgtgtgagtatgcacatccaggcaaaaggcactgaggtc
 cgggtgacagtgtctcgccaggtgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgaccttcta
 gatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactatccaaggactgagggccatggacacgggactctac
 atctgcaaggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatccagaaccgt

gcccagattctgatcagccaggttcctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccc
gactgtcactgcaccgaccggccctcgaggacctgtcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagat
gcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctac
agcgtgtccagtgctcctgccgggctgtgccgagccatggaacctgggaagacctcacttgcaactgctgcctaccccgagtccaag
5 accccgctaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccgtcggaggagctgg
ccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggctgcaggggtcacag
gagctgccccgcgagaagtacgtgactgggcatcccgagcaggagccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgctggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgtggccttcaca
cagaagaccatcgaccgcttgccgggtaaaccacccatgtcaatgtgtctgttgcacggcgagggtgactgataatctaga

10 AA

CTLA-4 IgAH IgA-T4 (amino acid sequence)

MGVLLTQRTLTLVLALLFPSMASMAMHVAQPAVVLAASSRGIASFVCEYASPGKAT
EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM
DTGLYICKVELMYPPYYLGIGNGTQIYVIDPEPCPDSDQPVPSTPPTPSPSTPPTSPS
15 CCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVFTFTWTPSSGKSAVQGPPDRD
LCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPP
PSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTTF
AVTSILRVAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAE
VD

20 NT

human IgG1 CH2 with 238 mutation pro→ser (nucleotide sequence)

cctgaactcctggggggatcgctcagtcttcttcccccaaaaccaaggacacccctcatgatctccggacccctgaggtcacat
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa
25 ggagtacaagtgcaaggtctccaacaagccctccagcccccatcgagaaaaccatctccaaagccaaag

AA

human IgG1 CH2 with 238 mutation pro→ser (amino acid sequence)

PELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

AA

Amino acids surrounding Pro to Ser in CH2 (amino acid sequence)

5 PAPELLGGPS

AA

Amino acids surrounding Pro to Ser in CH2 (amino acid sequence)

PAPELLGGSS

NT

10 **hIgE5Bcl (nucleotide sequence)**

ggt ggt gat cac gtc tgc tcc agg gac ttc acc cc

NT

hIgE3stop (nucleotide sequence)

ggt ggt tct aga tta act ttt acc ggg att tac aga cac cgc ctg g

15 NT

hIgE3BB (leaves an open reading frame at end of gene to read into transmembrane and cytoplasmic tail domain attached at either the BamHI or SfuI sites) (nucleotide sequence)

ggt ggt ttc gaa gga tcc gct tta ccg gga ttt aca gac acc gct cgc tgg

20 NT

human IgE Fc (CH2-CH3-CH4) ORF (nucleotide sequence)

tgatcacgtctgctccagggaacttcacccgcccaccgtgaagatcttacagtcgtctgcgacggcgggcgggcacttcccccgac
catccagctcctgtgcctcgtctctgggtacacccagggaactatcaacatcacctggctggaggacgggcaggtcatggacgtgg
acttgctccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacctcagccagaagcactggctgtca
25 gaccgcacctacactgccaggtcacctatcaagggtcacaccttgaggacagcaccaagaagtgtgcagattccaacccgagagg
ggtgagcgcctacctaagccggcccagccggtcgacctgttcacgcaagtcgccacgatcacctgtctggtggtggacctggc

acccagcaaggggaccgtgaacctgacctgggtcccgccagctgggaagcctgtgaaccactccaccagaaaggaggagaagc
 agcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcaggggggagacctaccagtgcaggg
 tgaccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgctgctgccccggaagtctatgcgtt
 tgcgacgccggagtggccggggagccgggacaagcgcacccctgcctgcctgatccagaacttcctgagggacatctcgggtg
 5 cagtggctgcacaacgaggtgcagctcccgagcggcgacagcacgacgagccccgcaagaccaagggtccgggttcttc
 gtcttcagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagcc
 cctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaagcggatccttcgaa

AA

human IgE Fc (CH2-CH3-CH4) ORF (amino acid sequence)

10 DHVCSRDFTPPTVKILQSSCDGGGHPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
 DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNP
 RGVSAYLSPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEK
 QRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYA
 FATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGF
 15 FVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

NT

IFhIgGwtBcl5 (nucleotide sequence)

gtt gtt tga tca gga gcc caa atc ttg tga caa aac tca cac atg ccc acc gtg ccc agc acc (63 mer)

NT

20 **hIgGWT3xba (nucleotide sequence)**

gtt gtt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g

NT

**HuIgGMHWC (sense, 5' primer for mutating wild type hinge CCC to mutant SSS)
(nucleotide sequence)**

25 gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa ctc ctg ggt
 gga ccg tca gtc ttc c

NT

1D8 VH (nucleotide sequence)

cagggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttcatta
accagcgaatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagat
tataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagtttcttaaaaatcaacagtctgcaaactga
5 tgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtcacagtctcctct

AA

1D8 VH (no leader) (amino acid sequence)

QVQLKEAGPGLVQPTQTLSTCTVSGFSLTSDGVHWIRQPPGKGLEWMGIYYDGG
TDYNSAIKSRLSISRDTSKSQVFLKINSLQTDDTAMYYCARIHFDYWGQGVMVTVS
10 S

NT

1D8 VL (no leader) (nucleotide sequence)

gacattgtgtcactcagtcctcaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaa
gttcatgtactggtaccagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatc
15 gcttcagtggcagtggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagt
ggagtagtactccgctcacgttcgggtctgggaccaagctggagatcaaacgg

AA

1D8 VL (amino acid sequence)

DIVLTQSPTTIAASPGKEKVTITCRASSSVSYMYWYQQKSGASPKLWIYDTSKLASGV
20 PNRFSGSGSGTSYSLAINTMETEDAATYYCQQWSSTPLTFGSGTKLEIKR

NT

1D8 scFv (nucleotide sequence)

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtcgttcagtcataatgtccagaggagtcgacattgtgtcactca
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagttacatgtactggtac
25 cagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
ggctctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgagtagtactccgc
tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatc

aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa
ccagcgatggtgtacactggattcgacagcctccaggaaaggtctggaatggatgggaataatatattatgatggaggcacagatt
ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagtctgcaaactgat
gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcacagtcacagtcctctgatca

5 AA

1D8 scFv (amino acid sequence)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS
STPLTFSGGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTVSG
10 FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS
LQTDDTAMYYCARIHFDYWQGVMVTVSS

NT

1D8 scFv IgG WTH WTCH2CH3 (nucleotide sequence)

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca
15 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagtacatgtactgttac
cagcagaagtcaggcgctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
ggctctgggacctcttattctctcgcaatcaacacatggagactgaagatgtgccacttattactgtcagcagtgaggtagtactccgc
tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtgggtgggtggcggcgatctc
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa
20 ccagcgatggtgtacactggattcgacagcctccaggaaaggtctggaatggatgggaataatatattatgatggaggcacagatt
ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagtctgcaaactgat
gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcacagtcacagtcctctgatcaggagcccaa
atcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagttctcttccccccaaaacc
aaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaa
25 ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcag
cgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcg
agaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatcccgggatgagctgaccaaga
accaggtcagcctgacctgcctggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaa
caactacaagaccacgcctcccgctgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggc

agcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagccctctccctgtctccggga
aatgatctaga

AA

1D8 scFv IgG WTH WTCH2CH3 (amino acid sequence)

5 MDFQVQIFSFLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS
STPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTVSG
FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAISKRLSISRDTSKSQVFLKINS
LQTDDTAMYYCARIHFDYWGGQVMVTVSSDQEPKSCDKTHTCPPCPAPPELLGGPS
10 VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK

NT

15 **1D8 scFv IgG MTH MTCH2CH3-CD80 (nucleotide sequence)**

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggtac
cagcagaagtcaggcgctccctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
ggctctgggacctcttattctctcgcaatcaacacatggagactgaagatgtgccacttattactgtcagcagtgaggtagtactccgc
20 tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatc
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa
ccagcgatggtgtacttgattcgacagcctccaggaaaggtctggaatggatgggaataatatattatgatggaggcacagatt
ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagctctgcaaactgat
gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa
25 atcttctgacaaaactcacacaagcccaccgagcccagcacctgaactcctggggggatcgtcagtccttcttccccccaaaaccc
aaggacacctcatgatctcccggaccttgaggtcacatgcgtgggtgggtggacgtgagccacgaagacctgagggtcaagttcaa
ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcag
cgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcg
agaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaaga

accaggtcagcctgacctgcctggcctcaaggcttctatcccagcgacatcgccgtggagtgaggagagcaatgggcagccggagaa
 caactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggc
 agcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggta
 aagcggatccttcgaacctgctccatcctgggccattacctaatactcagtaaatggaatgttgatgctgcctgacctactgctt
 5 gccccagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgata

AA

1D8 scFv IgG MTH MTCH2CH3-CD80 (amino acid sequence)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSVSVMYWY
 QKSGASPKLWIYDTSKLGSGVPSNRFGSGSGTSYSLAINTMETEDAATYYCQQWS
 10 STPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTVSG
 FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS
 LQTDDTAMYYCARIHFDYWGQGMVTVSSDLEPKSSDKTHTSPSPAPELLGGSSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLR
 15 PSRDELTKNQLVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSK
 LTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKADPSNLLPSWAITLISVN
 GIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

1D8 scFv IgG WTH WTCH2CH3-CD80 (nucleotide sequence)

20 aagcttatggatttcaagtgcagatttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtctccaacaaccatagctgcatctccaggggagaaggcaccatcacctgccgtgccagctccagtgaagttacatgtactggtag
 cagcagaagtcaggcgctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgagtagtactccgc
 tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcggcggtggtgggtggcggcggtggtcgc
 25 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagctctgcaactgat
 gacacagccatgtattactgtccagaatccacttgattactggggccaaggagtcagtggtcacagctcctctgatctggagcccaa
 atcttgtagaaaaactcacatgccaccgtgccagcacctgaactcctggggggaccgtcagcttctcttccccccaaaacc

aaggacaccctcatgatctccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaa
 ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcag
 cgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcg
 agaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaaga
 5 accaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaa
 caactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggc
 agcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggta
 aagcggatccttgaacctgctccatcctgggccattaccttaatctcagtaaatggaattttgtgatgctgcctgacctactgctt
 gccccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgata

10 AA

1D8 scFv IgG WTH WTCH2CH3-CD80 (amino acid sequence)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 QQKSGASPKLWIYDTSKSLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS
 STPLTFGSGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTVSG
 15 FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS
 LQTDDTAMYYCARIHFDYWQGVMVTVSSDLEPKSCDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYS
 20 KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPSWAITLISV
 NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

Anti-human CD3 scFv WTH WTCH2CH3 (nucleotide sequence)

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacac
 25 agactacatcctcctgtctgcctctctgggagacagagtcaccatcagttgcagggaagtcaggacattcgcaattatttaaactgg
 taticagcagaaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtcctcatcaaggttcagtggcag
 tgggtctggaacagattattctctcaccattgccaacctgcaaccagaagatattgccacttactttgccaacagggtataacgcttccg
 tggacgttcgggtggaggccacaaactggtaaccaaaccgggagctcgggtggcggtggctcgggcgggtgggtgggtcgggtggcg
 cggatctatcgatgaggtccagctgcaacagctctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggcctctggttac

tcattcactgggtacatcgtgaactgggtgaagcagagccatggaaagaaccttgagtggattggacttattaatccatacaaaggtctt
 actacctacaaccagaaattcaagggcaaggccacattaactgtagacaagtcacccagcacagcctacatggagctcctcagtctg
 acatctgaagactctgcagtctattactgtgcaagatctgggtactatgggtgactcggactggacttcgatgtctggggcgagggac
 cacggtcaccgtctcctctgatcaggagcccaaatctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggg
 5 gggaccgtcagctctcctcttcccccaaaaaccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtgggtggtgga
 cgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcggga
 ggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgc
 aaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtac
 accctgcccccatcccggtgatgactgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcccagcgacatcgc
 10 cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttctcctct
 acagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacca
 ctacacgcagaagagcctctccctgtctccgggtaaatgatctaga

AA

Anti-human CD3 scFv WTH WTCH2CH3 (amino acid sequence)

15 MDFQVQIFSFLISASVIMSRGVDIQMTQTSSLSASLGDRVTISCRASQDIRNYLNW
 YQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIANLQPEDIATYFCQQGN
 TLPWTFGGGTKLVTKRELGGGSGGGGSGGGGSIDEVQLQQSGPELVKPGASMSC
 KASGYSFTGYIVNWLKQSHGKNLEWIGLINPYKGLTTYNQKFKGKATLTVDKSSST
 AYMELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSSDQEPKSCDKT
 20 HTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

NT

25 **2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2h7-40.2.220Ig + restriction sites)**
(nucleotide sequence)

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaactcagtgcttcagtcataattgccagaggacaaattgttctctc
 ccagctcctcagcaatcctgtctgcatctccaggggagaaggtcaccaatgacttgaggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggtacctcccccaaacctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga

gtgggtctgggacctctactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggttttaac
ccaccacgttcggtgctgggaccaagctggagctgaaaggtggcgggtggctcgggcgggtggtgatctggaggaggtgggagc
tctcaggcttactacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcctgcaaggcttctggctacacat
ttaccagttacaatatgactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
5 tctacaatcagaagtcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaatccaactctgaagaagcaaagaaaggaggaggccaaaaaggagggaagccaagaaatctaacagc
gtcgacattgttctgactcagctccagccaccctgtctgtgactccaggagatagagtctctcttctgcagggccagccagagtatt
agcgactacttacactggtatcaacaaaatcacatgagtctccaaggcttctcatcaaatatgcttcccattccatctctgggatcccct
10 ccagggtcagtggcagtggtatcagggtcagatttactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaac
atggtcacagctttccgtggacgttcggtggaggcaccaagctggaaatcaaacgggggtggcgggtggctcgggcggaggtgggtc
gggtggcggcggatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaag
gcttctgggtatgccttcacaactactggaatgcagtgggtgcaagagatgccaggaaagggttggaatggattggctggataaac
acccactctggagtgcacaaaatgtagaagactcaaggacggttgcttctcttggaaacctctgcaaacactgcatatttacag
15 ataagcaacctcaaagatgaggacacggctactgtatttctgtgtgagatccgggaatggttaactatgacctggcctactttgcttactg
gggccaagggacactggtcactgtctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctga
actcctggggggatcgtcagcttctcttcccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc
cgcgaggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagta
20 caagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccaca
ggtgtacacctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcg
acatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcctt
cttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgca
caaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

25 AA

2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2H7-40.2.220Ig) (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN

MAVLGLLFCLVTFPSCVLSQVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWVR
QSPGKGLEWLGVWSSGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDAIYYC
ARNGGDNYPYYYAMDYWGQGTSVTVSS

5 **5B9 VH** (no leader peptide) (amino acid sequence)

QVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSSGI
TDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDAIYYCARNGGDNYPYYYAMDY
WGQGTSVTVSS

NT

10 **5B9 VL** (nucleotide sequence)

atgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgcaggctgcattctcca
atccagtcactcttgaacatcagcttccatctcctgcaggctagtaagagtctcctacatagtaatggcatcacttattgtattggtatc
tgcagaagccaggccagctcctcagctcctgattatcagatgtccaacctgcctcaggagtcagacaggttcagtagcagtg
gtcaggaactgattcacactgagaatcagcagagtgaggctgaggatgtgggtgttattactgtgctcaaaatctagaactccgct
cacgttcggtgctgggaccaagctggagctgaaacgg

AA

5B9 VL (amino acid sequence)

MRFSAQLLGLLVLPWGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHSNGITYL
YWYLQKPGQSPQLLIYQMSNLA SGVPDRFSSSGSGTDFTLRISRVEAEDVG VYYCA
20 QNLELPLTFGAGTKLELKR

NT

5B9 scFv (nucleotide sequence)

aagcttgccgcatgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgcag
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggctagtaagagtctcctacatagtaatggcatcactta
25 ttgtattggtatctgcagaagccaggccagctcctcagctcctgattatcagatgtccaacctgcctcaggagtcagacaggttc
agtagcagtggtcaggaactgattcacactgagaatcagcagagtgaggctgaggatgtgggtgttattactgtgctcaaaatct
agaactccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcggtgggtgggtcgggtg
cggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagacctgtccatcacctgcacagctt

ctggtttctcattaactacctatgctgtacactgggttcgccagctctccaggaaagggctctggagtggctgggagtgatatggagtgg
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttcttaaaatgaacagt
ctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa
ggaacctcagtcaccgtctctct

5 AA

5B9 scFv (amino acid sequence)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSNGITYL
YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGYYCA
QNLELPLTFGAGTKLELKRGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT
10 CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV
FFKMNSLQPNDTAIYYCARNGGDNPYYYAMDYWGQGTSVTVSS

NT

5B9 scFv-hmtIgG1-hCD80 (nucleotide sequence)

aagcttgccgccatgaggttctctgctcagcttctggggctgctgtgctctggatccctggatccactgcagatattgtgatgacgcag
15 gctgcattctccaatccagtcactcttgaacatcagcttccatctctgcaggtctagtaagagtctctacatagtaatggcatcactta
tttgattggtatctgcagaagccaggccagctctctcagctcctgatttatcagatgtccaaccttgccctaggagtcccagacaggttc
agtagcagtgggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgttttactgtgctcaaaatct
agaacttccgtcacgttcggtgctgggaccaagctggagctgaaacgggggtggcggtggctcgggcggtgggtgggtcgggtgg
cggcggtatctcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagctct
20 ctggtttctcattaactacctatgctgtacactgggttcgccagctctccaggaaagggctctggagtggctgggagtgatatggagtgg
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttcttaaaatgaacagt
ctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa
ggaacctcagtcaccgtctctctgatctggagcccaatcttctgacaaaactcacacaagcccaccgagcccagcacctgaactc
ctggggggatctcagcttctcttccccccaaaaccaaggacacctcatgatctcccggacctgaggtcacatgcgtggtg
25 gtggacgtgagccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgc
gggaggagcagtacaacagcacgtacctgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa
gtgcaaggtctccaacaaagccctcccagcccccacgagaaaacatctccaaagccaaagggcagccccgagaaccacaggt
gtacacctgcccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgaca
tcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgacggctcctctt

cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcaca
 accactacacgcagaagagcctctccctgtctccggtaaagcggatcctcgaacctgctcccatcctgggccattacctaatactca
 gtaaatggaattttgtgatatgctgcctgacctactgctttgcccccaagatgcagagagagaaggaggaatgagagattgagaaggg
 aaagtgtacgccctgtataaatcgataactcgag

5

AA

5B9 scFv-hmtIgG1-hCD80 (amino acid sequence)

MRFSAQLLGLLVLPWPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHNSGITYL
 YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA
 10 QNLELPLTFGAGTKLELKRGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT
 CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV
 FFKMNSLQPNDAIYYCARNGGDNYPIYYAMDYWGQGSVTVSSDLEPKSSDKTH
 TSPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
 VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
 15 KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKAD
 PSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

20 NT

2e12 scFv WTH CH2 CH3 (2e12 scFv-WthIgG-CD80) (nucleotide sequence)

aagcttatggatttcaagtcagattttcagcttctgctaatacagtcgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagttta
 atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgccagg
 25 tttagtggcagtggtctgggacagactcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
 aggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggt
 ggcggcggtatcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaaatgaa

cagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatggtatggactactggggtcaa
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttgacaaaactcacacatgccaccgtgccagcacctgaactc
 ctggggggaccgtcagtcctcctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgctggtg
 gtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgc
 5 gggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa
 gtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt
 gtacacctgccccatcccgggatgagctgaccaagaaccagggtcagcctgacctgctgtcaaaggcttctatcccagcgaca
 tcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttct
 cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcaca
 10 accactacagcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattacctaatactca
 gtaaatggaattttgtgatatgctgcctgacctactgctttgccccagatgcagagagagaaggaggaatgagagattgagaaggg
 aaagtgtacgccctgtataaatcgat

AA

2e12 scFv WTH CH2 CH3 (2e12 scFv-WthIgG-CD80) (amino acid sequence)

15 MDFQVQIFSFLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL
 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ
 QSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLITC
 TVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDGSTDYNSALKSRLSITKDNSKSQ
 VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQGSVTVSSDLEPKSCDKT
 20 HTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKA
 DPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

25 NT

2H7-human IgE Fc (CH2-CH3-CH4) (nucleotide sequence)

aagcttgcgcctatggattttcaagtgcagatttcagcttctgctaactcagtgcttcagtcataattgccagaggacaaattgttctctc
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcaccaatgacttgaggccagctcaagtgttaagttacatgcactgg
 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga

gtgggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggagtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagggtggcgggtggctcgggcgggtggtgatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggctcttggtacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
5 tctacaatcagaagtcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctctgatcacgtctgtccagggaacttccccgccaccgtgaagatcttacagtcgtcctgcgacggcggcgggca
cttcccccgaccatccagctcctgtgcctcgtctctgggtacacccagggaactatcaacatcacctggctggaggacgggcaggt
catggagctggacttgtccaccgcctctaccacgcaggaggggtgagctggcctccacacaaagcagagtcaccctcagccagaag
10 cactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaagtgtgcagattcc
aaccgagaggggtgagcgctacctaagccggcccagccggttcgacctgttcacccgaagtcggccacgatcacctgtctggt
ggtggacctggcaccagcaaggggaccgtgaacctgacctgggtccgggccaagtgggaagcctgtgaaccactccaccagaaa
ggaggagaagcagcgcaatggcacgttaaccgtcacgtccacctgccgggtgggcacccgagactggatcgagggggagacct
accagtgcagggtgaccacccccacctgccaggggccctcatgcggtccacgaccaagaccagcgccccgcgtgctgccccg
15 gaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcacctcgcctgcctgatccagaacttcatgcctg
aggacatctcgggtgcagtggctgcacaacgaggtgcagctcccgagcggcgacagcacgacgcagccccgaagaccaag
gggtccggcttctctgcttccagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcacat
gaggcagcgagccccacagaccgtccagcgagcgggtgtctgtaaatcccgtaaatgataatctaga

AA

20 **2H7 scFv IgE (CH2-CH3-CH4) (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
25 QLSSLTSEDSAVYFCARVVYYSNSYWFVDVWGTGTTVTVSDHVCSDRFTPTVKIL
QSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELAST
QSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSPSPFDLFI
RKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGT
RDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLA

CLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQK
DEFICRAVHEAASPSQTVQRAVSVNPGK

NT

2H7 scFv MH (SSS) MCH2WTCH3 (nucleotide sequence)

5 aagcttgccgcatggattttcaagtgagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
cccccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctggcggtggtggatctggaggaggtgggagc
10 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcgtcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaggagcccaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga
15 tcgtcagttcttcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga
gccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtacctgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggctc
tccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg
ccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatccagcgacatcgccgtgga
20 gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctggtgactccgacggctccttcttctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg
cagaagagccctcctctcctccgggtaaatgatctaga

AA

2H7 scFv MH (SSS) MCH2WTCH3 (amino acid sequence)

25 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP

SPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

5 NT

5B9 scFv MTHWTCH2CH3 (nucleotide sequence)

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgcag
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta
tttgattggtatctgcagaagccaggccagctctcctcagctcctgatttatcagatgtccaacctgcctcaggagtccagacaggttc
10 agtagcagtgggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctcaaaatct
agaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcgggtgggtgggtcgggtgg
cggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtct
ctggtttctcattaactacctatgctgtacactgggttcgccagctctccaggaaagggctgagtggtgggagtgtatggagtgg
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt
15 ctgcaacctaatgacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa
ggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcc
tggggggaccgtcagcttctccttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgctggtgg
tggacgtgagccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaag
20 tgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgt
acacctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

25

AA

5B9 scFv MTHWTCH2CH3 (amino acid sequence)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSHNGITYL
YWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA
QNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT
5 CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV
FFKMNSLQPNDAIYYCARNGGDNYPIYYAMDYWGQGTSTVTVSSDQEPKSSDKT
HTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
10 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

Human IgG1 hinge mutations

NT

2H7 scFv- MTH (CSS) WTCH2CH3 (nucleotide sequence)

Nucleotide:

15 aagcttgccgccatggattttcaagtgcagatttcagcttctgtaatacgtgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
gtgggtctgggacctctactcttcacaatcagcagagtgaggagctgaagatgctgccactattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
20 tctcaggcttatctacagcagctctggggtgagctggtaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
ttaccagttacaatatgcactgggtaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtgatact
tcctacaatcagaagttcaagggaagccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttactgggtacttcgatgtctggggcacagggaccac
gggtaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggg
25 accgtcagctcttctctccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtgggtgggtgacgtg
agccacgaagaccctgagggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggag
cagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggt
ctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaaggcagccccgagaaccacaggtgtacacct
gccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccagcgacatgccgtgg

agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagc
aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
gcagaagagcctctccctgtctccgggtaaatgatctaga

AA

5 **2H7 scFv- MTH (CSS) WTCH2CH3 (amino acid sequence):**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
10 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDKTHTSPP
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPP
VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

15 NT

2H7 scFv- MTH (SCS) WTCH2CH3 (nucleotide sequence)

aagcttgccgccatggattttcaagtcagatttcagcttctgctaatacagtcgttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgtctgcttcagtgga
20 gtgggtctgggaccttctactcttcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
25 atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttactggtacttcgatgtctggggcacaggaccac
ggtcaccgtctcttctgatcaggagcccaatcttctgacaaaactcacacatgccaccgtccccagcacctgaactcctggggggg
accgtcagcttcttcttcccccaaaaccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtg
agccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggag
cagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggt

ctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacct
gcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcccagcgacatcgccgtgg
agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctacagc
aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
5 gcagaagagcctctccctgtctccgggtaaatgatctaga

AA

2H7 scFv- MTH (SCS) WTCH2CH3 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
10 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTCPP
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
15 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP
VLDSGDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

NT

2H7 scFv- MTH (SSC) WTCH2CH3 (nucleotide sequence)

aagcttcccgccatggattttcaagtgcagatttcagcttctgctaatacgtgcttcagtcataattgccagaggacaaattgttctctc
20 ccagcttccagcaatctgtctgcattctccaggggagaaggtcacatgacttgcaggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggtgagctggtagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
25 ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact
tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgaagagtgggtactatagtaactcttactgggtacttcgatgtctggggcacaggaccac
ggtcaccgtctcttctgatcaggagccaaatctctgacaaaactcacacatccccaccgtgccagcacctgaactcctggggggg
accgtcagctcttcttctccccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtg

agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggag
cagtacaacagcacgtaccgtgtggcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggt
ctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacct
gcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtgg
5 agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagc
aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
gcagaagagcctctccctgtctccgggtaaatgatctaga

AA

2H7 scFv- MTH (SSC) WTCH2CH3 (amino acid sequence)

10 MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP
15 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

NT

20 **HIgGMHcys1 (nucleotide sequence)**

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tg

NT

HIgGMHcys2 (nucleotide sequence)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tct cca ccg tgc

25 NT

HIgGMHcys3 (nucleotide sequence)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tcc cca gca cct

NT

HuIgG1 MTCH3Y405 (nucleotide sequence)

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctg
gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccc
5 gtgctggactccgacggctccttctacatctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgc
tccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

AA

HuIgG1 MTCH3Y405 (amino acid sequence)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
10 VLDSDGsfYLYSKLTVDKSRWQQGNVfSCSVMHEALHNHYTQKSLSLSPGK

NT

HuIgG1 MTCH3A405 (nucleotide sequence)

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctg
gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccc
15 gtgctggactccgacggctccttcgccctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatg
ctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

AA

HuIgG1 MTCH3A405 (amino acid sequence)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
20 VLDSDGsfALYSKLTVDKSRWQQGNVfSCSVMHEALHNHYTQKSLSLSPGK

NT

HuIgG1 MTCH3A407 (nucleotide sequence)

Gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
gggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcc
25 cgtgctggactccgacggctccttcttctcgcagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcat
gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

AA

HuIgG1 MTCH3A407 (amino acid sequence)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDS DGSFFLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

5 **HuIgG1 MTCH3Y405A407 (nucleotide sequence)**

gggcagccccgagaaccacaggtgtacacctgcccccatccgggaggagatgaccaagaaccaggtcagcctgacctgctg
gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccc
gtgtggactccgacggctccttctacctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatg
ctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

10 AA

HuIgG1 MTCH3Y405A407 (amino acid sequence)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDS DGSFY LASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

15 **HuIgG1 MTCH3A405A407 (nucleotide sequence)**

gggcagccccgagaaccacaggtgtacacctgcccccatccgggaggagatgaccaagaaccaggtcagcctgacctgctg
gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccc
gtgtggactccgacggctccttcgccctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatg
ctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

20 AA

HuIgG1 MTCH3A405A407 (amino acid sequence)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDS DGSFALASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

25 **2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (nucleotide sequence)**

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagtacatgactgg

taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca
gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggaggctcgggcgggtggatctggaggaggtgggagc
tctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacat
5 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga
ccgtcagtcttcttctccccccaaaaccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga
10 gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc
tccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
ccccatcccgaggagatgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatccagcgacatcgccgtgg
agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttctacctctatagc
15 aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
gcagaagagcctctccctgtccccgggtaaatgatctaga

AA

2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
20 QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVYYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
25 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
PVLDSGDSFYLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

NT

2H7 scFv MTH (SSS) WTCH2MTCH3A405 (nucleotide sequence)

aagcttgccgcatggattttcaagtgcagattttcagcttctgtaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgaagttacatgcactgg
 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca
 gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 5 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
 tctcaggcttatctacagcagcttggggctgagctggtagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
 tcctacaatcagaagtcaagggcaaggccacactgactgtagacaaatcctccagcagacgctacatgcagctcagcagcctgac
 atctgaagactctcggtctatttctgtgcaagagtgggtactatagtaactttactggtacttcgatgtctggggcacaggggaccac
 10 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga
 ccgtcagctcttcttctccccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgggacgtga
 gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
 agtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggct
 tccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg
 15 ccccatcccgggaggagatgaccaagaaccaggtcagcctgacctggtcaaaggcttctatccagcgacatgccgtgg
 agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttcgcctctatag
 caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactaca
 cgcagaagagcctctccctgtccccgggtaaatga

AA

20 **2H7 scFv MTH (SSS) WTCH2MTCH3A405 (nucleotide sequence)**

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasnlsgvparfsg
 sgshtsysltisrveadaatyycqqsfnpptfgagtkleldggsggggssgggssqaylqqsgaelvrpgasvkmsckas
 gytftsynmhwwkqtrprglewigaiypngdtsynqkfkkatltvdkssstaymqlssltedsavyfcarvvysnsywy
 fdvwgtgtvtvssdqepkssdkthtspspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv
 25 evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsreemtknqvslt
 clvkgfypsdiavewesngqpennykttppvldsdgsfalyskltdksrwqqgnvfscsvmhealhnhytqkslslspgk

NT

2H7 scFv MTH (SSS) WTCH2MTCH3A407 (nucleotide sequence)

aagcttgccgcatggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagtctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagttacatgcactgg
 taccagcagaagccaggatcctcccccaccctggatttatgcccataccacctggcttctggagtcctgctcgttcagtgga
 gtgggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
 5 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatggtgatact
 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcagacacctacatgcagctcagcagcctgac
 atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaccac
 10 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga
 ccgtcagcttctcttccccccaaaaccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga
 gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
 agtacaacagcagctaccgtgtggtcagcgtcctaccgtctgcaccaggactgggtgaatggcaaggagtacaagtgaaggct
 tccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg
 15 ccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatgccgtgg
 agtgggagagcaatgggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctcgcag
 caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactaca
 cgcagaagagcctctccctgtccccgggtaaatga

AA

20 **2H7 scFv MTH (SSS) WTCH2MTCH3A407 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
 QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
 PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
 25 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP
 SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
 PVLDSGDSFFLASKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

NT

2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (nucleotide sequence)

aagcttgccgcatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgaagtacatgcactgg
5 taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgtcgcttcagtggca
gtgggtctgggaccttctactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
tctcaggcttatctacagcagcttggggctgagctggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
10 tctacaatcagaagtcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaggagcccaaattcttgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggga
ccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgctggtggtggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
15 agtacaacagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtc
tccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
ccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtgg
agtgggagagcaatgggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctacctgccag
caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactaca
20 cgcagaagagcctctccctgtccccgggtaaatga

AA

2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSPGGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
25 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVYYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA

KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP
PVLDSGDSFYLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (nucleotide sequence)

5 aagcttgccgccatggattttcaagtcagattttcagcttctgctaatacagtcgttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggaagtttaac
cccccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctggcggtgggtgatctggaggaggtgggagc
10 tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaagatgctcgaaggcttctgggtacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtatact
tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga
15 ccgtcagttctctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtgggtgggtgacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggct
tccaacaaagccctcccagccccatcgagaaaacaatctccaagccaaaggcgagccccgagaaccacaggtgtacaccctg
ccccatccccgggaggagatgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgacatcgccgtgg
20 agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcgccctcgcca
gcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactac
acgcagaagagcctctccctgtccccgggtaaatga

AA

2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (amino acid sequence)

25 MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP

SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
PVLDSDSGSFALASKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK

5 NT

2H7 scFv MTH (SCC) WTCH2CH3 (nucleotide sequence)

aagcttgccgccatggattttcaagtgcagatttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttcagggccagctcaagtgaagttacatgcactgg
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
10 gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtgctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat
ttaccagttacaatatgactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
15 atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggaccac
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatgccaccgtgccagcacctgaactcctgggggg
accgtcagcttctcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtg
agccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggag
cagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaagg
20 ctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaaggcagccccgagaaccacaggtgtacacct
gccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccagcgacatcgccgtgg
agtgggagagcaatgggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagc
aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
gcagaagagcctctccctgtctccgggtaaatgatctaga

25 AA

2H7 scFv MTH (SCC) WTCH2CH3 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSPGGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG

YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
 QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTCPP
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 5 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP
 VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

NT

2H7 scFv MTH (CSC) WTCH2CH3 (nucleotide sequence)

aagcttgccgccatggattttcaägtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
 10 ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga
 gtgggtctgggacctctactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 ccaccacggtcgggtctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtgatctggaggaggtgggagc
 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat
 15 ttaccagttacaatatgcactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
 atctgaagactctgcggctctattctgtgcaagagtgggtgactatagtaactcttacttggtacttcgatgtctggggcacagggaccac
 ggtcaccgtctctctgatcaggagcccaaatctgtgacaaaactcacacatctccaccgtgccagcacctgaactcctgggggga
 ccgtcagctctctcttccccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtga
 20 gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc
 tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctg
 cccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccagcgacatcgccgtgga
 gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagca
 25 agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacag
 cagaagagcctctccctgtctccgggtaaatgatctaga

AA

2H7 scFv MTH (CSC) WTCH2CH3 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHYQ
 QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
 PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
 5 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDKTHTSPP
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPP
 VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

10 NT

2H7 scFv MTH (CCS) WTCH2CH3 (nucleotide sequence)

aagcttgcgcatgatttcaagtcagatttcagctcctgctaatactgctcagtcataattgccagaggacaaattgtctctc
 ccagctccagcaatcctgtctgcattccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagtacatgactgg
 taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga
 15 gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtgctcgggcgggtggtgatctggaggaggtgggagc
 tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat
 ttaccagttacaatatgactgggtaagcagacacccatgacagggcctggaatggattggagctattatccaggaaatggtgatact
 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcagacccatcatgcagctcagcagcctgac
 20 atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttactggtacttcgatgtctggggcacagggaccac
 ggtcaccgtctctctgatcaggagcccaaatctgtgacaaaactcacacatgtccaccgtccccagcacctgaactcctgggggga
 ccgtcagcttctcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtgggtgggacgtga
 gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtc
 25 tccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
 ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtgga
 gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctggtgactccgacggctccttctctctacagca
 agctcaccgtggacaagagcaggtggcagcaggggaacgtctctcatgctccgtgatgcagaggctctgcacaaccactacacg
 cagaagagcctctccctgtctccgggtaaatgatctaga

AA

2H7 scFv MTH (CCS) WTCH2CH3 (amino acid sequence)

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
5 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDKTHTCPP
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
10 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP
VLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

HuIgAHIgA-T4-ORF (nucleotide sequence)

tgatcagccagttccctcaactccactaccccatctccctcaactccactaccccatctccctcatgctgccaccccgactgtcact
15 gcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg
tcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgtacagcggtcca
gtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgctgcctaccccgagtccaagaccccgcta
accgccacccctcctcaaatccggaacacattccggcccgaggccacctgctgccgccgccgtcgaggagctggccctgaacg
agctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccc
20 cgcgagaagtacctgacttgggcatcccgagcaggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgtgg
cagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcacacagaagacca
tcgaccgcttgccgggtaaacccacccatgtcaatgtgtctgttgcacgaggtggacgcggatccttcgaac

AA

HuIgAHIgA-T4-ORF (amino acid sequence)

25 DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVT
FTWTPSSGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP
REKYLTWASRQEPSQGTTTFAVTSILRVA AEDWKKGDTFSCMVGHEALPLAFTQKT
IDRLAGKPTHVNVSVVMAEVDADPSN

NT

HuIgAHIgA-T4-ORF (nucleotide sequence)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcact
gcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg
5 tcaccticacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgtacagcggtcca
gtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgtgcctaccccagtgccaagaccccgcta
accgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccgtcggaggagctggccctgaacg
agctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgcc
cgcgagaagtacgtgacttgggcatcccgccaggagcccgccagggcaccaccaccttcgctgtgaccagcatactgcgcgtgg
10 cagccgaggactggaagaagggggacaccttctcctgcatggtgggcccacgaggccctgccgtggccttcacacagaagacca
tcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcacatggcggaggtggacgcggatccttcgaac

AA

HuIgAHIgA-T4-ORF (amino acid sequence)

DQPVPSTPPTSPSTPPTSPSCCHPRLSLHRPALEDLLGSEAILTCTLTGLRDASGVT
15 FTWTPSSGKSAVQGPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP
REKYLTWASRQEPSQGTTFVAVTSILRVA AEDWKKGDTFSCMVGHEALPLAFTQKT
IDRLAGKPTHVNVSVVMAEVDADPSN

NT

20 **1D8-IgAH IgA-T4-CD80 (nucleotide sequence)**

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactggtac
cagcagaagtgcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagt
ggctctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgagtagtactccgc
25 tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtgggtgggtggcggcggtatctc
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa
ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaaatcaacagctctgaaactgat
gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcacgtgtcctctgatcagccagttcc

ctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcactgcaccgaccggcc
 ctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctgga
 cgccctcaagtgggaagagcgctgttcaaggaccacctgacctgtacctctgtggctgctacagcgtgtccagtgtcctgccgggct
 gtgccgagccatggaacctgggaagaccttcactgcactgctgcctaccccgagtccaagaccccgctaaccgccaccctctca
 5 aaatccggaacacattccggcccagggtccacctgctgccgccgcccgtcgaggagctggccctgaacgagctggtgacgctg
 acgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccccgcgagaagtacct
 gactgggcatcccgagcaggagccagggcaccaccaccttcgctgtgaccagcactgcgcgtggcagccgaggactg
 gaagaagggggacaccttctctgcatggtggggcacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcg
 ggtaaacccacctatgtcaatgtgtctgttgcctggcgagggtggacgcgatcctcgaacaacctgctcccatctggggccatta
 10 ccttaatctcagtaaatggaattttgtgatatgctgcctgacctactgctttgccccaatgagagagagaaggaggaatgagaga
 ttgagaagggaagtgtacgcctgtataaatcgatac

AA

1D8 scFv IgAH IgA-T4-CD80 (amino acid sequence)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 15 QQKSGASPKLWIYDTSKLASGVNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS
 STPLTFSGGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTVSG
 FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS
 LQTTDDTAMYYCARIHFDYWQGVMVTVSSDQVPSTPPTPSPSTPPTPSPSCCHPRL
 SLHRPALEDLLLSEAILTCTLTGLRDASGVFTWTPSSGKSAVQGPDRDLGCYS
 20 VSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPPPSEELAL
 NELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTFFAVTSILR
 VAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAEVDADPS
 NNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

25 human IgE Fc (CH2-CH3-CH4) ORF (nucleotide sequence)

tgatcacgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcgggcgggcacttcccccgac
 catccagctcctgtgcctcgtctcgggtacacccagggactatcaacatcacctggctggaggacgggcaggtcatggacgtgg
 acttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacctcagccagaagcactggctgtca
 gaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattccaacccgagagg

ggtgagcgcctacctaagccggcccagcccgttcgacctgttcacccgaagtcgcccacgatcacctgtctggtggtgacctggc
 acccagcaaggggaccgtgaacctgacctggccccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagc
 agcgcaatggcacgttaaccgtcacgtccaccctgccgggtgggcacccgagactggatcgagggggagacctaccagtgcaggg
 tgaccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcgccccgcgtgctgccccggaagtctatgcgtt
 5 tgcgacgccggagtggccggggagccgggacaagcgcaccctcgctgcctgatccagaacttcatgcctgaggacatctcggtg
 cagtggctgcacaacgaggtgcagctcccgacgcccggcacagcacgacgcagccccgaagaccaagggtccggcttcttc
 gtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagcc
 cctcacagaccgtccagcgagcgggtgtctgtaaatcccggtaaagcggatccttcgaa

AA

10 **human IgE Fc (CH2-CH3-CH4) ORF (amino acid sequence)**

DHVCSDFTPTVKILQSSCDGGGHPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
 DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNP
 RGVSAYLSRSPFDLFIKRSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEK
 QRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYA
 15 FATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSF
 FVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

NT

1D8 scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)

aagcttatggattttcaagtgcagattttcagcttcctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgtcactca
 20 gtctccaacaaccatagctgcattctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaattacatgtactggtac
 cagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgtagtactccgc
 tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtgggtggcggcgatctc
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcatgactgtctctgggttctcattaa
 25 ccagcgatggtgtactggttcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttctaaaaatcaacagctcgaaaactgat
 gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcaggtcacagtctcctctgacacgtctgctcc
 agggacttcaccccggccaccgtgaagatcttacagtcgtcctgcgacggcggcggcacttcccccgacctccagctcctgtg
 cctcgtctctgggtacaccccagggaactatcaacatcacctggctggaggacgggcaggtcatggacgtggacttgccaccgcctc

taccacgcaggaggggtgagctggcctccacacaaagcgagctcacctcagccagaagcactggctgtcagaccgcactacac
 ctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaagtgtgcagattccaacccgagaggggtgagcgcctacc
 taagccggcccagcccgttcgacctgttcacgcaagtcgccacgatcacctgtctgggtggacctggcaccagcaagggg
 accgtgaacctgacctggctccggggcagtggggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcaatggcac
 5 gttaacctgcacgtccaccctgccgggtgggcacccgagactggatcgagggggagacctaccagtgcagggtgacccaccccca
 cctgccaggggccctcatcggtccacgaccaagaccagcgccccgcgtgctgccccggaagtctatgcgtttcgacgccgga
 gtggccggggagccgggacaagcgcaccctcgctgcctgatccagaactcatgcctgaggacatctcgggtgcagtggctgcac
 aacgaggtgcagctcccgagcggcgccgacagcacgacgcagccccgcaagaccaagggctccggcttctctcagccgc
 ctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagccccctacagacc
 10 gtccagcgagcgggtgtctgtaaattcccggtaaagcggatccttcgaagctcccatcctgggccattaccttaattcagtaaatggaat
 tttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacg
 ccctgtataaatcgata

AA

1D8-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)

15 MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGKVTITCRASSSVSYMYWY
 QKSGASPKLWIYDTSKLASGVNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS
 STPLTFGSGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTVSG
 FSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS
 LQTTDDTAMYYCARIHFDYWQGQVMVTVSSDHVCSRDFTPPTVKILQSSCDGGGHF
 20 PPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLSQKH
 WLSDRITYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSPSPFDLFIKSPITCLV
 VDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETY
 QCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMP
 DISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVH
 25 EAASPSQTVQRAVSVNPGKADPSKLPSWAITLISVNGIFVICCLTYCFAPRCRERRRN
 ERLRRESVRPV

NT

5B9-IgAH IgA-T4-CD80 (nucleotide sequence)

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgcag
 gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggcttagtaagagtctctacatagtaatggcatcactta
 tttgtattggtatctgcagaagccaggccagctcctcagctcctgatttatcagatgtccaaccttgccctcaggagctccagacaggctc
 agtagcagtgggtcaggaactgattcacactgagaatcagcagagtgaggctgaggatgtgggtgtttattactgtgctcaaaatct
 5 agaacttccgctcacgttcggtgctgggaccaagctggagctgaaacgggggtggcgggtggctcgggcgggtgggtgggtcgggtgg
 cggcggatcgtcacagggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtct
 ctggtttctcattaactacctatgctgtacactgggttcgccagctctccaggaaagggtctggagtggctgggagtgatatggagtgg
 ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagat
 ctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa
 10 ggaacctcagtcaccgtctcctctgatcagccagttccctcaactccactaccccatctccctcaactccactaccccatctccctca
 tctgtccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgacc
 ggctgagagatgcctcagggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacct
 ctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcactgactgctgcctac
 cccgagtccaagaccccgtaccgccacctctcaaaatccgaaacacattccggcccgaggtccacctgctgccgccgccgt
 15 cggaggagctggccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggct
 gcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccgagcaggagcccagccagggcaccaccaccttcg
 ctgtgaccagcactactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgc
 cgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccaccatgtcaatgtgtctgttcatggcggaggtggacg
 cggatccttcgaacaacctgctccatcctgggccattaccttaatctcagtaaatggaattttgtgatatgctgcctgacctactgctt
 20 gccccagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgatac

AA

5B9-IgAH IgA-T4-CD80 (amino acid sequence)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSNGITYL
 YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA
 25 QNLELPLTFGAGTKLELKRGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLIT
 CTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSKSQV
 FFKMNSLQPNDAIYYCARNGGDNPYYYAMDYWGQGTSVTVSSDQVPVPSTPPTP
 SPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWPSSGKS
 AVQGPPDRDLGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNT

FRPEVHLLPPPSEELALNELVTLTCLARGFSPKDLVRWLQGSQELPREKYLTWASR
QEPSQGTTTTFAVTSILRVA AEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTH
VNVSVVMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRR
ESVRPV

5 NT

5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)

aagcttgccgcatgaggttctctgctcagcttctggggctgctgtgctctggatccctggatccactgcagatattgtgatgacgcag
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtcctacatagtaatggcatcactta
tttgattggtatctgcagaagccaggccagctcctcagctcctgattatcagatgtccaacctgcctcaggagctccagacaggttc
10 agtagcagtgggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgtttactgtgctcaaaatct
agaacttccgtcacgttcggtgctgggaccaagctggagctgaaacggggtggcgggtggctcgggcggtgggtgggtcgggtgg
cggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtct
ctggtttctcattaactacctatgctgtacactgggttcgccagctcctcaggaaagggtctggagtggctgggagtatatggagtgg
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt
15 ctgcaacctaatgacacagccatttactgtgccagaaatgggggtgataactaccttattactatgctatggactactggggtaa
ggaacctcagtcaccgtctcctctgatcacgtctgtccaggacttcacccgccaccgtgaagatcttacagtcgtcctgcgacg
gcggcgggcacttcccccgacctccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggag
gacgggcaggtcatggactggactgtccaccgccttaccacgcaggagggtgagctggcctccacacaaagcgagctcaccc
tcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaagggtcacaccttgaggacagcaccaagaag
20 tgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgaagtcgccacgat
cacctgtctggtggaggacctggcaccagcaaggggaccgtgaacctgacctgggtcccgggagctgggaagcctgtgaaccac
tccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccacctgccggtgggcacccgagactggatcgag
ggggagacctaccagtgcagggtgacccacccccacctgccaggggcctcatgcggtccacgaccaagaccagcgggccgcg
tgctgccccggaagtctatgcgttgcgacgccggagtggccggggagccgggacaagcgaccctgcctgcctgatccagaac
25 ttcatgcctgaggacatctcgggtgcagtgggtgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgc
aagaccaagggtccggcttctcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccg
tgagtcctcatgaggcagcgagccctcacagaccgtccagcgagcgggtgtctgtaaatcccggtaaacgggatccttcgaagctcc
catcctgggccattaccttaatctcagtaaatggaattttgtgatatgctgcctgacctactgcttgccccaagatgcagagagagaag
gaggaatgagagattgagaagggaagtgtagccctgtataaatcgata

AA

5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSNGITYL
YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA
5 QNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT
CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV
FFKMNSLQPNDAIYYCARNGGDNYPIYYAMDYWGQGTSVTVSSDHVCSRDFTPP
TVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEG
ELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAAYLSRPS
10 PFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTS
TLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSR
DKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRA
EWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISVNGIFVICCL
TYCFAPRCRERRRNERLRRESVRPV

15 NT

2e12-scFv-IgAH IgA-T4-CD80 (nucleotide sequence)

aagcttatggatttcaagtcagatttccagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctcagagccagtgaaagtgtgaatattatgcacaagtta
atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgccagg
20 tttagtggcagtggtctgggacagactcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattctgtcagcaaagt
aggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggt
ggcggcgggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct
caggggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaaatgaa
25 cagctctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactggggtcaa
ggaacctcagtcaccgtctcctcagatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccctca
tgtgtccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgacc
ggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctgacct
ctgtgggtgtacagcgtgtccagtgctctgccgggtgtgccgagccatggaacctgggaagacctcactgtcactgtgcctac

cccgagtccaagacccccgtaaccgccaccctctcaaaatccggaaacacattccggcccagggtccacctgctgccgccgct
 cggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggct
 gcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcg
 ctgtgaccagcactactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgc
 5 cgctggccttcacacagaagaccatcgaccgcttggcggttaaaccacccatgtcaatgtgtctgttgcaggggaggtggacg
 cggatccttcgaacaacctgctcccatcctgggccattaccttaatctcagtaaattgaattttgtgatgtgctgacctactgctt
 gccccaatgagcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgatac

AA

2e12-scFv-IgAH IgA-T4-CD80 (amino acid sequence)

10 MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL
 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ
 QSRKVPWTFGGGTKLEIKRGGGGSGGGSGGGGSQVQLKESGPGLVAPSQSLITC
 TVSGFSLTGYGVNWVRQPPGKLEWLGMWGDGSTDYNSALKSRLSITKDNSKSQ
 VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWQGTSVTVSSDQVPSTPPTP
 15 SPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWTPSSGKS
 AVQGPPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNT
 FRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASR
 QEPSQGTTTTFAVTSILRVAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTH
 VNVSVMMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRR
 20 ESVRPV

NT

2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)

aagcttatggattttcaagtgcagattttcagcttctgctaatacgtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaagtta
 25 atgcagtgttaccacagaaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggtcctgccagg
 tttagtggcagtggtctgggacagactcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgcagcaaagt
 aggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggtggcggtggctcgggcggaggtgggtcgggt
 ggcggcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg

atggaagcacagactataattcagctctcaaattccagactgagcatcaccaaggacaactccaagagccaagttttctaaaaatgaa
 cagtctgcaaactgatgacacagccagatactactgtgccagagatggttagtaactttcattactatgttatggactactggggtaa
 ggaacctcagtcaccgtctcctcagatcacgtctgtccagggaactcaccgccaccgtgaagatcttacagtcgtcctgcgacg
 gcggcgggcaactccccccgaccatccagctcctgtgcctcgtctctgggtacaccccagggaactatcaacatcacctggctggag
 5 gacgggcaggtcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacc
 tcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaag
 tgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcacccgcaagtcgccacgat
 cacctgtctggtggtggacctggcaccagcaaggggaccgtgaacctgacctgggtccgggaccagtggaagcctgtgaaccac
 tccaccagaaaggaggagaagcagcgcgaatggcacgttaaccgtcacgtccacctgccggtgggacccgagactggatcgag
 10 ggggagacctaccagtgcaggggtgacccacccccacctgccaggggccctcatgcggtccacgaccaagaccagcggcccg
 tgtgtcccccgaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaac
 ttcagcctgaggacatctcgggtgcagtggtgcacaacgaggtgcagctcccgacgccgggcacagcacgacgcagccccgc
 aagaccaagggtccggcttctcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccg
 tgcagtcctgaggcagcgagccccctcacagaccgtccagcgagcgggtgtctgtaaatcccggtaaagcggatccttcgaagctcc
 15 catcctgggccattaccttaatctcagtaaatggaattttgtgatatgtgcctgacctactgctttgccccagatgcagagagagaag
 gaggaatgagagattgagaagggaagtgtacgccctgtataaatcgata

AA

2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL
 20 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ
 QSRKVPWTFGGGTKLEIKRGGGGSGGGSGGGGSQVQLKESGPGLVAPSQSLITC
 TVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNSKSQ
 VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWQGTSVTVSSDHVCSRDFTP
 PTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEG
 25 ELASTQSELTLQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPS
 PFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTS
 TLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSR
 DKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRA

EWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISVNGIFVICCL
TYCFAPRCRERRRNERLRRESVRPV

NT

500A2 scFv (nucleotide sequence)

5 atgttgatacatctcagctccttgggcttttactcttctggatttcagcctccagaagtacatagtgctgactcagactccagccactct
gtctctaattcctggagaaaagagtcacaatgacctgtaagaccagtcagaatattggcacaatcttacctgggtatcaccaaaaaccaa
aggaggctccaagggtctcatcaagtatgcttcgagtcattcctgggatccctccagattcagtggcagtggttcggaacaga
tttactctcagcatcaataacctggagcctgatgatacgggaatttactgtcaacaaagtagaagctggcctgtcacgttcggtcct
ggcaccaagctggagataaaacgggggtggcggtggctcgggcggaggtgggtggcggcggtatcaggtcaagctgca
10 gcagtcgggttctgaactagggaaacctggggcctcagtgaactgtcctgcaagacttcaggctacatattcacagatcactatattt
cttgggtgaaacagaagcctggagaaagcctgcagtgataggaaatgtttatgggtgaaatggtgtacaagctacaatcaaaaatt
ccagggaaggccacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgaggattctgccat
ctattactgtgcaagaaggccggtagcgacgggccatgctatggactactggggtcaggggatccaagttaccgtctcctctgac

AA

15 **500A2 scFv (amino acid sequence)**

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYHQ
KPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPVTF
GPGTKLEIKRGGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIFTD
HYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMELSSLT
20 SEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSD

NT

5' oligo:

Name : hIgAbcl5

Sequence : GTTGTTGATCAGCCAGTTCCTCAACTCCACCTACC

25 NT

3' oligo:

Name : IgA3BB

GTTGTTTTCGAAGGATCCGCGTCCACCTCCGCCATGACAACAGA

NT

5' oligo:

Name : IgGWT3

5 GTTGTTTTCGAAGGATCCGCTTTACCCGGAGACAGGGAGAGGCTCTT

NT

3' oligo:

Name : hIgGWT5

GTTGTTAGATCTGGAGCCCAAATCTTGTGACAAAACCTCACACATG

10 NT

5' oligo:

Name : FADD5

Sequence :

GTTGTGGATCCTTCGAACCCGTTCTGGTGCTGCTGCACTCGGTGTGCG

15 NT

3' oligo:

Name : FADD3

Sequence :

GTTGTTATCGATCTCGAGTTATCAGGACGCTTCGGAGGTAGATGCGTC

20 NT

FADD-CSSCFV (nucleotide sequence)

gtggatccttgaacccgttcctggtgctgctgactcgggtgctccagcctgtcagcagcgagctgaccgagctcaagttcctat
gcctcgggcgcggtgggcaagcgcaagctggagcgcggtcagagcggcctagaccttctccatgctgctggagcagaacgacc
tgagagccgggacacccgagctcctgcgcgagctgctgcctccctgcggcgccacgacctgctcgggcgcgctgacgacttcg
25 aggcggggggcgggcgccggggcgccgctggggaagaagacctgtgtgcagcatttaacgtcatatgtgataatgtggggaag
attggagaaggctggctcgtcagctcaaagtctcagacaccaagatcgacagcatcgaggacagataaccccgcaacctgacaga
cgctgtgcgggagtcactgagaatctggaagaacacagagaaggagaacgcaacagtggcccacctggtgggggctctcaggtc

ctgccagatgaacctgggtggctgacctggtacaagaggttcagcaggcccgtagacctccagaacaggagtggggccatgtccccg
atgtcatggaactcagacgcatctacctccgaagcgtcctgataactcgagatcgataacaac

AA

FADD-CSSCFV (amino acid sequence)

5 VDPSNPFLVLLHSVSSSLSSSELTFLKFLCLGRVVGKRLERVQSGLDLFSMLLEQND
LEPGHTELLRELLASLRRHDLRRVDDFEAGAAAGAAPGEEDLCAAFNVICDNVVGK
DWRRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRIWKNTOKENATVAHLVGALR
SCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

NT

10 **HCD28tm5B (nucleotide sequence)**

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGTGGTTGGTGTCTGGCTTGCTATA
GCTTG

NT

HCD28tm3S (nucleotide sequence)

15 GTTGTTTCGAACCCAGAAAATAATAAAGGCCACTGTTACTAGCAAGCTATAGCA
AGCCAG

NT

HCD28tm5' (nucleotide sequence)

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGT

20 NT

HCD28tm3' (nucleotide sequence)

GTTGTTTCGAACCCAGAAAATAATAAAGGCCAC

NT

HCD80tm5' (nucleotide sequence)

25

GTTGTGGATCCTCCTGCTCCCATCCTGG

NT

HCD80tm3' (nucleotide sequence)

GTTGTTTCGAACGGCAAAGCAGTAGGTCAGGC

NT

5 **MFADD5BB (nucleotide sequence)**

GTTGTGGATCCTTCGAACCCATTCTGGTGCTGCTGCACTCGCTG

NT

MFADD3XC (nucleotide sequence)

GTTGTTATCGATCTCGAGTCAGGGTGTTTCTGAGGAAGACAC

10 NT

Murine FADD nucleotide sequence (full length, but without flanking -Ig or transmembrane sequences) (nucleotide sequence)

gtggatccttcgaacatggaccattcctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatctgatggagctcaagt
tcttggtccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacggtgctgctggagcagaa
15 cgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgtgcgccgacacgatctactgcagcgcctggacga
cttcgaggcggggacggcgaccgctgcgggggggggaggcagatctgcaggtggcatttgacattgtgtgtgacaatgtgggg
agagactggaaaagactggcccgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccgaagtctg
agtgagcgggtaaggagagtgctgaaagtctggaagaatgctgagaagaagaacgcctcggtgccggactggtcaaggcgctg
cggacctgcaggctgaatctggtggctgacctggtggaagaagcccaggaatctgtgagcaagagtgagaatatgtccccagtact
20 aagggattcaactgtgtcttctcagaaacaccctgactcgagatcgat

AA

Murine FADD (amino acid sequence)

VDPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDLFTVLLE
QNDLERGHTGLLRELLASLRRHDLLQRLDDFEAGTATAAPPGEADLQVAFDIVCDN
25 VGRDWKRLARELKVSEAKMDGIEEKYPRSLSERVRESLKVWKNAEKKNASVAGL
VKALRTCRLNLVADLVEEAQESVSKSENMSPVLRDSTVSSSETP

NT

MCASP3-5 (nucleotide sequence)

GTTGTGGATCCTTCGAACATGGAGAACAACAAAACCTCAGTGGATTCA

NT

MCASP3-3 (nucleotide sequence)

5 GTTGTTATCGATCTCGAGCTAGTGATAAAAGTACAGTTCTTTCGT

NT

MCASP8-5 (nucleotide sequence)

GTTGTTTCGAACATGGATTTCCAGAGTTGTCTTTATGCTATTGCTG

NT

10 **MCASP8-3 (nucleotide sequence)**

GTTGTTATCGATCTCGAGTCATTAGGGAGGGAAGAAGAGCTTCTTCCG

NT

hcasp3-5(nucleotide sequence)

GTTGTGGATCCTTCGAACATGGAGAACACTGAAAACCTCAGTGGAT

15 NT

hcasp3-3 (nucleotide sequence)

GTTGTTATCGATCTCGAGTTAGTGATAAAAATAGAGTTCTTTTGTGAG

NT

hcasp8-5 (nucleotide sequence)

20 GTTGTGGATCCTTCGAACATGGACTTCAGCAGAAATCTTTATGAT

NT

hcasp8-3 (nucleotide sequence)

GTTGTTATCGATGCATGCTCAATCAGAAGGGAAGACAAGTTTTTTTCT

NT

25 **HuIgGMHWC (nucleotide sequence)**

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa ctc ctg ggt
gga ccg tca gtc ttc c

NT

2H7-human IgE (CH2-CH3-CH4) (nucleotide sequence)

5 aagcttgccgccatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagcttccagcaatcctgtctgcatctccaggggagaaggcacaatgacttgcaaggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
cccccacgttcggctgtgggaccaagctggagctgaaagggtggcgggtggctcggcggtggatctggaggaggtgggagc
10 tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtgatact
tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggctctatttctgtgcaagagtgggtgactatagtaacttactgtgacttcgatgtctggggcacagggaccac
ggtcaccgtctctgatcacgtctgctccagggaacttcaccccgccaccgtgaagatcttacagtcgtcctgcgacggcgggcgggca
15 cttccccccgaccatccagctcctgtgcctcgtctctgggtacaccccagggaactatcaacatcacctggctggaggacgggcaggt
catggacgtggacttgtccaccgcctctaccacgcaggaggggtgagctggcctccacacaaagcgagctcacctcagccagaag
cactggctgtcagaccgcacctacacctgccagggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattcc
aaccggagaggggtgagcgcctacctaaagccggcccagcccgttcgacctgttcacccgaagtcgcccacgatcacctgtctggt
gggtggacctggcaccagcaaggggacctgaacctgacctggctccggggccagtggaagcctgtgaaccactccaccagaaa
20 ggaggagaagcagcgcaatggcacgttaacctgcacgtccacctgccgggtgggcacccgagactggatcgagggggagacct
accagtgcaagggtgacccacccccacctgccaggggccctcatgcgggtccacgaccaagaccagcggcccgcgtgctgccccg
gaagtctatcggttgcgacgccggagtggccggggagccgggacaagcgcacccctcgctgcctgatccagaacttcatgcctg
aggacatctcgggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagaccaag
ggctccggcttctctgcttccagccgcctggaggtgaccagggccgaatgggagcagaaaagatgagttcatctgccgtgcagtccat
25 gaggcagcgagccccctcacagaccgtccagcgagcgggtgtctgtaaatcccgtaaatgataatctaga

AA

2H7 scFv IgE (CH2-CH3-CH4) (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN

PPTFGAGTKLELKGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
 QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSDHVCSDFTPTVKIL
 QSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELAST
 5 QSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSPSPFDLFI
 RKSPITITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGT
 RDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLA
 CLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQK
 DEFICRAVHEAASPSQTVQRAVSVNPGK

10 NT

2H7 scFv MH (SSS) MCH2WTCH3 (nucleotide sequence)

aagcttgccgccatggattttcaagtcagatttcagcttcctgctaatacagtcagtcataattgccagaggacaaattgtctctc
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacatgactgcaggccagctcaagtgaagtacatgactgg
 taccagcagaagccaggatcctcccccaccctggatttatgcccacccaacctggcttctggagtcctgctgcttcagtgga
 15 gtgggtctgggacctcttactctctcacatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggtttaac
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc
 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcgaagcttctggctacacat
 ttaccagttacaatatgactgggtaagcagacacccagcagggcctggaatggattggagctatttatccaggaaatggtgatact
 tcctacaatcagaagtcaagggaagggcacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
 20 atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttactggacttcgatgtctggggcacaggaccac
 ggtcaccgtctctctgatcaggagcccaatctctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga
 tcgtcagcttctctctccccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtggacgtga
 gccacgaagaccctgaggtcaagtcaactggtagtgacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggctc
 25 tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctg
 ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatccagcgacatcgccgtgga
 gtgggagagcaatgggcagccggagaacaactacaagaccagcctcccgctggactccgacggctccttctctctacagca
 agctcaccgtggacaagagcaggtggcagcaggggaacgtctctcatgctccgtgatgcatgaggtctgcacaaccactacacg
 cagaagagcctctccctgtctccgggtaaatgatctaga

AA

2H7 scFv MH (SSS) MCH2WTCH3 (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ
QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN
5 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSP
SPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
10 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
VLDSGDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

NT

5B9 scFv MTHWTCH2CH3 (nucleotide sequence)

aagcttgccgcatgaggttctctgctcagcttctgggctgcttgtgctctggatccctggatccactgcagatattgatgacgcag
15 gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta
tttgattggtatctgcagaagccaggccagctcctcagctcctgatttatcagatgtccaacctgcctcaggagtcccagacaggttc
agtagcagtgggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgttattactgtgctcaaatct
agaacttccgtcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcggcggtgggtgggtcgggtgg
cggcggtatgtcacaggtgcagctgaagcagtcaggacctggcctagtcagtcctcacagagcctgtccatcacctgcacagtct
20 ctggtttctcattaactacctatgctgtacactgggttcgccagcttccaggaaaggtctggagtggctgggagtatatggagtgt
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt
ctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggggtcaa
ggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
tggggggaccgtcagttctcctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgg
25 tggacgtgagccacgaagacctgaggtcaagtcaactgttacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactgggtgaatggcaaggagtacaag
tgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgt
acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaggcttctatcccagcgacatc
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttcttc

tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

AA

5B9 scFv MTHWTCH2CH3 (amino acid sequence)

5 MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHNSNGITYL
YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA
QNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT
CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV
FFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSTVTVSSDQEPKSSDKT
10 HTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

15 **1. 2H7 scFv with alternative VHL11 mutations:**

Nucleotide sequence

Aagcttgccgccatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcatctccaggggagaaggtcaccaatgacttgcagggccagctcaagtgttaagttacatgactgg
taccagcagaagccaggatctccccaaacctggattatgccccatccaacctggcttctggagtcctgctcgttcagtgga
20 gtgggtctgggacctctactctctcaccaatcagcagagtgaggagctgaagatgctgccacttattactgccagcagtgaggatttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggtgag (one of the following: ten, acn, gan, can, aan, cgn, agn)
gtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacatttaccagttacaatatgactgggtaaagcagacac
ctagacagggcctggaatggattggagctattatccaggaaatggtgatacttctacaatcagaagttcaagggaaggccacact
25 gactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcggctctatttctgtgcaagagt
gtgtactatagtaactcttactgtgacttcgatgtctggggcacagggaccacgggcaccgtctcttctgatcag

Amino acid sequence

mdfqvqifsflisaviiargqivlsqspailsaspgkvmtncrasssvsymhwyqqkpgsspkpwiapsnlasgvparfsg
30 ssgtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgae (one of the
following: s, t, d, e, q, n, r, k, h)
vrpgasvkmsckasgytftsynmhwwkqtrpglewigaiypngdtsynqkfkkgkatltvdksstaymqllslltsedsav
fcarvvyysnsywyfdvwgtgtvtvssdq

35 **2. VHL11 deletion**

Nucleotide sequence:

Aagcttgccgcatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 5 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagc
 tctcaggcttatctacagcagctctggggctgaggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacatta
 ccagttacaatatgcactgggtaaagcagacacactagacagggcctggaatggattggagctatttatccaggaaatggtgatacttc
 ctacaatcagaagttaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacat
 ctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccacgg
 10 tcaccgtctcttctgatcag

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiapsnlasgvparsfsg
 sgshtsysltisrveadaatyccqwsfnpptfgagtklekdggsgggsgggssqaylqqsgaevrpgasvkmsckas
 15 gyftsynmhwwkqtrpqlgwigaiypngdtsynqkfkglktltdkssstaymqlssltedsavyfcarvvyysnsywy
 fdvwgtgtvtvssdq

3. 2H7 VL L106 with alternative mutations

Nucleotide sequence:

20 aagcttgccgcatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 ccaccacgttcggtgctgggaccaagctggag (tcn, agn, aan, cgn, can, gan, and non-natural
 25 derivatives of these codons) aaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagctc

Amino acid sequence:

Mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiapsnlasgvparsfsg
 sgshtsysltisrveadaatyccqwsfnpptfgagtkle (s, t, r, k, h, q, n, d, e, and non-natural
 30 derivatives of these amino acids at position 106)kdggsgggsgggsgss

4. VL L106 deletion

Nucleotide sequence:

35 Aagcttgccgcatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 ccaccacgttcggtgctgggaccaagctggagaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagctc
 40

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiapsnlasgvparsfsg
 sgshtsysltisrveadaatyccqwsfnpptfgagtklekdggsgggsgggsgss

45 5. IgE CH3 CH4

Nucleotide sequence:

tccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcacccgcaagtcgcccacgatcacctgtct
 ggtggtggacctggcaccagcaaggggaccgtgaacctgacctggtcccgggaccagtgggaagcctgtgaaccactccaccag
 aaaggaggagaagcagcgcgaatggcacgttaacctgacgtccaccctgccggtgggcacccgagactggatcgagggggaga
 cctaccagtgcagggtgacccacccccacctgcccaggccctcatgcggtccacgaccaagaccagcggcccgcgtgtctgcc
 5 cggaaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcc
 tgaggacatctcggtgcagtggtgcacaacgaggtgcagctcccggacggcggcacagcacgacgcagccccgaagacca
 agggctccggcttcttcgtttcagccgcctggaggtgaccaggccgaatgggagcagaaagatgagttcatctgccgtgcagtc
 catgaggcagcagccccctcacagaccgtccagcgagcgggtgtctgtaaaccggtaaatgataatctagaa

10 Amino acid sequence:
 snprgvsaylsrpspfdlfrksptitclvdlapskgtnltwsrasgkpvnhstrikeekqrmgtltvtstlpvgtrdwiegetyqcr
 vthphlpralmrstktsgraaapevyafatpewpgsrdrklaciqlfmpedisvqwlhnevqlpdarhsttqprktksgffv
 fsrlevtraeweqkdeficravheaaspsqtvqravsvnpgk

15 6. hIgG1H/IgE WCH3 WCH4

Nucleotide sequence:

tgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcatccaacccgagaggggtgagcgcctaccta
 gccggcccagcccgttcgacctgttcacccgcaagtcgcccacgatcacctgtctggtggtggacctggcaccagcaaggggac
 cgtgaacctgacctggtcccgggaccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcgaatggcacgtt
 20 aaccgtcacgtccacctgccggtgggcacccgagactggatcgagggggagacctaccagtgcagggtgacccacccccacct
 gccagggccctcatgcggtccacgaccaagaccagcggcccgctgctgccccggaagtctatgcgtttgcgacgccggagt
 gccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcctgaggacatctcggtgcagtggtgcacaac
 gaggtgcagctcccgacggcggcacagcagcagccccgaagaccaagggtccggcttcttcgtttcagccgcctg
 gaggtgaccaggggcgaatgggagcagaaagatgagttcatctgcctgacgtccatgaggcagcagccccctcacagaccgtc
 25 cagcgagcgggtgtctgtaaaccggtaaatgataatctagaa

Amino acid sequence:

dqepkssdkthtspspasnpgrgvsaylsrpspfdlfrksptitclvdlapskgtnltwsrasgkpvnhstrikeekqrmgtltvt
 stlpvgtrdwiegetyqcrvthphlpralmrstktsgraaapevyafatpewpgsrdrklaciqlfmpedisvqwlhnevqlp
 30 darhsttqprktksgffvfsrlevtraeweqkdeficravheaaspsqtvqravsvnpgk

7. IgE WCH2 WCH3 WCH4

Nucleotide sequence:

Tgatcacgtctgtccagggaacttccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttccccga
 35 ccatccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggaggacgggcaggtcatggacgtg
 gacttgtccaccgcctctaccacgcaggaggggtgagctggcctccacacaaagcagctcacctcagccagaagcactggctgt
 cagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccagaaggtgtgcagattccaaccgaga
 ggggtgagcgcctacctaagccggcccagcccgttcgacctgttcacccgcaagtcgcccacgatcacctgtctggtggtggacct
 ggcaccagcaagggggaccgtgaacctgacctggtcccgggaccagtgggaagcctgtgaaccactccaccagaaaggaggaga
 40 agcagcgcgaatggcacgttaacctgacgtccaccctgccggtgggcacccgagactggatcgagggggagacctaccagtga
 gggtgacccacccccacctgcccaggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccggaagtctatg
 cgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcctgaggacatctc
 ggtgcagtggtgcacaacgaggtgcagctcccggacggcggcacagcacgacgcagccccgaagaccaagggtccggc
 ttcttcgtttcagccgcctggaggtgaccaggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctatgaggcagc
 45 gagccccctcacagaccgtccagcgagcgggtgtctgtaaaccggtaaatgataatctaga

Amino acid sequence:

dhvcsrdftptvkilqsscdggghfpptiqlclvsytpgtinitwledgqvmddvlstasttqegelastqseltlsqkhwlslrt
yticvtyqghtfedstkkcadsnprgvsaylsrpspfdlfrksptitclvvdlapskgtnltwsrasgkpvnhstrkeekqrmgtl
tvtstlpvgtrdwiegetyqcrvthphlpralmrsttktsgraaapevyafatpewpgsrdrklaciqnfmpeidisvqwlhnev
5 qlpdarhsttqprktkgsffvfrlevtraeweekdeficravheaaspsqtvqravsvnpgk

8. hIgG1H/IgE CH3 CH4 (ORF)

Nucleotide sequence:

Nucleotide sequence:

10 tgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcatccaacccgagaggggtgagcgcctaccta
gcccggccagcccgttcgacctgttcacccgcaagtcgcccacgatcacctgtctggtggtggacctggcaccagcaaggggac
cgtgaacctgacctggtcccgggccagtgggaagcctgtgaacctccaccagaaaggaggagaagcagcgaatggcacgtt
aaccgtcacgtccacctgccggtgggcacccgagactggatcgagggggagacctaccagtgcagggtgacccacccccac
gcccagggccctcatcggtccacgaccaagaccagcggcccgctgctgccccggaagtctatcggttgcgacgccggagt
15 gccggggagccgggacaagcgcacccctgcctgcctgatccagaacttcacgtgaggacatctcggtgcagtggctgcacaac
gaggtgcagctcccgagcggcgacacgcagcagcagccccgaagaccaagggctccggcttcttcgtctcagccgcctg
gaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctgaggcagcagccctcacagaccgtc
cagcgagcgggtgtctgtaaatcccggtaaagcggatcctcga

20 Amino acid sequence:

dqepkssdkthtspspasnpgrvsaylsrpspfdlfrksptitclvvdlapskgtnltwsrasgkpvnhstrkeekqrmgtltvt
stlpvgtrdwiegetyqcrvthphlpralmrsttktsgraaapevyafatpewpgsrdrklaciqnfmpeidisvqwlhnevqlp
darhsttqprktkgsffvfrlevtraeweekdeficravheaaspsqtvqravsvnpgksgsfe

25 9. 2H7 VHL11S scFv hIgG1(SSS-S)H hIgE WCH3 WCH4

Nucleotide sequence:

aagcttccgcatggattttcaagtcagatttccagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatctgtctgcattctccaggggagaagggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg
taccagcagaagccaggtatctccccaaacctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtggca
30 gtgggtctgggaccttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatggtgatact
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
35 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttctactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaggagcccaatcttctgacaaaactcacacatccccaccgtcctcagcatccaacccgagaggggt
gagcgcctacctaagccggcccagcccgttcgacctgttcacccgaagtcgcccacgatcacctgtctggtggtggacctggcac
ccagcaaggggaccgtgaacctgacctggtcccggccagtggaagcctgtgaacctccaccagaaaggaggagaagcag
cgcaatggcacgttaacctcacgtccacctgccggtgggcacccgagactggatcgagggggagacctaccagtgcagggtg
40 accacccccacctgccagggccctcatgcggtccacgaccaagaccagcggcccgctgctgccccggaagtctatgcgttg
cgacgccggagtggccggggagccgggacaagcgcacccctgcctgcctgatccagaacttcacgtgaggacatctcggtgc
agtggctgcacaacgaggtgcagctcccgagcggcgacacgcagcagccccgcaagaccaagggctccggcttcttc
gtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctgaggcagcagcc
cctcacagaccgtccagcgagcgggtgtctgtaaatcccggtaaatgataatctaga

45

Amino acid sequence:

Mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfs
 gsgsgtsysltisrveaadaatyycqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsck
 asgytfts ynmhvwkqtrpqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvysnsy
 wyfdvwtgtttvtvssdqepkssdkthtspssasnprgvsaylsrpspfdlfrksptitclvvdlapskgtnltwsrasgkpv
 5 nhstrkeekqrmgtltvtstlpvgrdwiegetyqcrvthphlpralmrsttktsgpaaapevyafatpewpgsrdrkrtlacliqnfm
 pedisvqwlhnevqlpdarhsttqprktkgsffvfrlevtraeweqkdeficravheaaspsqtvravsvnpgk

10. 2H7 VHL11S scFv hIgG1(SSS-P)H hIgE WCH3 WCH4

10 Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgaggccagctcaagtgttaagtacatgcaactgg
 taccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
 gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 15 ccaccacggttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
 tctcaggcttatctacagcagcttgaggctgagtcggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
 ttaccagttacaatatgcaactgggtaaagcagacacctagacagggcctggatggattggagctattatccaggaaatggtgatact
 tctacaatcagaagttaagggaaggccacactgactgtagacaaatctccagcagacgctacatgcagctcagcagcctgac
 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacaggggaccac
 20 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcatccaacccgagaggggt
 gagcgctacctaagccggccagcccgttcgacctgttcacgcaagtcgcccacgatcacctgtctggtggtggacctggcac
 ccagcaaggggaccgtgaacctgacctgggtcccgggccagtgagggaagcctgtgaaccactccaccagaaaggaggagaagcag
 cgcaatggcacgttaaccgtcacgtccaccctgccggtgggacccgagactggatcagggggagacctaccagtgagggtg
 accacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggccgcgtgctgccccggaagtctatgcgtttg
 25 cgacgccggagtggccggggagccgggacaagcgcacctcgcctgcctgatccagaacttcagctgaggacatctcgtgac
 agtgggtgcacaacgaggtgcagctcccgacggccgacagcagcagcagccccgaagaccaaggggtccggcttcttc
 gtcttcagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctatgaggcagcgagcc
 cctcacagaccgtccagcgagcgggtgtctgtaaatcccggtgaatgataatctaga

30 Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
 ssgsgtsysltisrveaadaatyycqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
 gytfts ynmhvwkqtrpqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvysnsywy
 fdvwtgtttvtvssdqepkssdkthtspssasnprgvsaylsrpspfdlfrksptitclvvdlapskgtnltwsrasgkpv
 35 trkeekqrmgtltvtstlpvgrdwiegetyqcrvthphlpralmrsttktsgpaaapevyafatpewpgsrdrkrtlacliqnfmpe
 disvqwlhnevqlpdarhsttqprktkgsffvfrlevtraeweqkdeficravheaaspsqtvravsvnpgk

10. 2H7 VL L106S

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 40 ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgaggccagctcaagtgttaagtacatgcaactgg
 taccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
 gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 ccaccacggttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
 tc

45

Amino acid sequence:

mdfqvqifsflisaviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveadaatyycqqsfnpptfgagtkleskdggsgggsgggss

5 **11. 2H7 VL L106S scFv**

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctcagcaatcctgtctgcatctccaggggagaaggcacaatgacttgcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtggca
10 gtgggtctgggacctctactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggtttaac
ccaccacgttcgggtgctgggaccaagctggagctctaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
15 atctgaagactctgcggctctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcag

Amino acid sequence:

mdfqvqifsflisaviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
20 sgsgtsysltisrveadaatyycqqsfnpptfgagtkleskdggsgggsgggssqaylqqsgaelvrpgasvkmsckas
gytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvvysnsywy
fdvwgtgtvtvssdq

25 **12. 2H7 scFv VL L106S VHL11S scFv**

Nucleotide sequence:

Aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctcagcaatcctgtctgcatctccaggggagaaggcacaatgacttgcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtggca
30 gtgggtctgggacctctactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggtttaac
ccaccacgttcgggtgctgggaccaagctggagctctaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggctctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
35 ggtcaccgtctcttctgatcag

Amino acid sequence:

mdfqvqifsflisaviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveadaatyycqqsfnpptfgagtkleskdggsgggsgggssqaylqqsgaesvrpgasvkmscka
40 sgytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvvysnsywy
yfdvwgtgtvtvssdq

45 **10. Human IgD hinge linker with attached restriction sites**

Nucleotide:

gtggatccaggttcgaagtctccaaaggcacaggcctcctccgtgccactgcacaaccccaagcagagggcagcctcgccaagg
caaccacagccccagccaccaccgtaacacaggaagaggaggagaagagaagaagaaggagaaggagaagaggaacaag
aagagagagagacaaagaccggtgcagtcgacg

- 5 Amino acid:
vdpgskspkaqassvptaqpqaegslakattapattmntgrggeekkkkekeeeqeeretktgavd

Sequence of Native IgD hinge domain:

(includes a cysteine residue—we truncated the hinge prior to that residue for these
10 constructs:)

Nucleotide:

gagtctccaaaggcacaggcctcctccgtgccactgcacaaccccaagcagagggcagcctcgccaaggcaaccacagcccca
gccaccaccgtaacacaggaagaggaggagaagagaagaagaaggagaaggagaaagaggaacaagaagagagagagac
aaagacaccagagtgtccgagccacaccagcctcttggcgtctacctgtaaccct

- 15 Amino acid sequence:
Espkaqassvptaqpqaegslakattapattmntgrggeekkkkekeeeqeeretktpecpshtqplgvyltp

12. 2H7 VH L11S

- 20 Nucleotide sequence:
caggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacattta
ccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctattatccaggaaatggtgatacttc
ctacaatcagaagtcaaggcgcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacat
ctgaagactctgcggtctattctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccacgg
25 tcaccgtctcttct

Amino acid sequence:
qaylqqsgaesvrpgasvkmckasgytftsynmhvkwqtprrglewigaiypngdtsynqkfkkgkatltvdkssstaym
qlssltsedsavyfcarvvyysnsywyfdvwtgtvtvss

- 30
13. 2H7 VH L11S scFv
Nucleotide sequence:
aagcttgccgccatggattttcaagtgcagatttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcacatccaggggagaagggtcacaatgacttgcagggccagctcaagtgttaagttacatgcactgg
35 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggttttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctattatccaggaaatggtgatact
40 tctacaatcagaagtcaaggcgcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctattctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcag

- Amino acid sequence:
45 mdfqvqifsflisasviiargqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveadaatyycqqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmckas

gytftsynmhvwkqtrprglewigaiypngdtsynqkfkgkatltvdkssstaymqlssltsedsavyfcarvvyyssnsywy
fdvwgtgtvtvssdq

5 **14. 2H7 scFv VH L11S hIgG1 (CSC-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca
10 gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtgatctggaggaggtgggagc
tctcaggcttatctacagcagtctggggctgagctctgtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacatt
taccagttacaatatgcactgggtaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatactt
cctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcagcctacatgcagctcagcagcctgaca
15 tctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaaccacg
gtcaccgtctcttctgatcaggagcccaaatcttgacaaaactcacacatctccaccgtgctcagcacctgaactcctgggtggacc
gtcagctcttctcttcccccaaaacccaaggacacctcatgatctccggaccttgaggtcacatgcgtggtggtggacgtgag
ccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
gtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctt
20 ccaacaagccctccagccccatcgagaaaacaatctcaaagccaaaggcagccccgagaaccacaggtgtacacctgc
ccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccaagcgacatcgccgtggag
tgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaa
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagggctctgcacaaccactacacgc
agaagagcctctcctgtctccggtaaatgatctaga

25 Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg
sgsgtsysltisrveadaatyycqqsfnpptfgagtkleldggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsynmhvwkqtrprglewigaiypngdtsynqkfkgkatltvdkssstaymqlssltsedsavyfcarvvyyssnsywy
30 fdvwgtgtvtvssdqepkscdkthtsppcsapellggpsvflfppkpkdtlmisrtpcvvvdvshedpevkfnwyvdgv
evhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapietiskakgqprepvytlppsrdeltknqvsltc
lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhlealnhhtqkslsispkg

15. 2H7 scFv VH L11S IgE WCH2 WCH3 WCH4

35 Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca
40 gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtgatctggaggaggtgggagc
tctcaggcttatctacagcagtctggggctgagctctgtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacatt
taccagttacaatatgcactgggtaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatactt
cctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcagcctacatgcagctcagcagcctgaca
tctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaaccacg
45 gtcaccgtctcttctgatcacgtctgctccagggacttcacccgccaccgtgaagatcttacagtcgtcctgcacggcgggcggg
acttccccccgaccatccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggaggacgggcagg

tcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacctcagccagaa
 gcaactggctgtcagaccgcacacacctgccaggtcacctatcaaggtcacacctttgaggacagcacaagaagtgtgcagattc
 caaccggagaggggtgagcgccctacctaagccggccagccggttcgacctgttcacccgaagtcgcccacgatcacctgtctgg
 5 tgggtggacctggcaccagcaaggggaccgtgaacctgacctggctccggggccagtgggaagcctgtgaaccactccaccagaa
 aggaggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgagggggagacc
 taccagtgcagggtgacccacccacacctgccagggccctcatcggtccacgaccaagaccagcggcccgctgctgccccg
 gaagtctatcggttgcgacgccggagtggccggggagccgggacaagcgacccctcgctgctgatccagaacttcacgtcgtg
 aggacatctcggtgcagtggctgcacaacgaggtgcagctccggagcggcgacagcacgacgcagccccgaagaccaag
 ggctccggctcttcgtcttcagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcacat
 10 gaggcagcgagccccctcacagaccgtccagcgagcgggtgtctgtaaattcccgtaaataatgataatctaga

Amino acid sequence:

mdfvqqlfllisaviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg
 ssgtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
 15 gytftsynmhwwkqtrpqlgwigaiypngdtsynqkfkkgkatltvdksstaymqlssltedsavvyfcarvvyysnsywy
 fdvwtgtttvtssdhvcsrdftptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmddlstatteqegelastq
 seltslqkhwlsdrtytcqvtqgthfedstkkcadsnprgvsaylsrpsfdlfrksptitclvdlapskgtvnlwsrasgkpv
 nhstrkeekqrmgtltvtstlpvgrdwiegetyqcrvthphlpralmrsttktspraapevyafatpewpgsrdrklaciqnfm
 pedisvqwlhnevqlpdarhsttqprktksggffvfrlevtraeweqkdeficravheaaspsqtvravsvnpgk

16. 2H7 scFv VH L11S mIgE WCH2 WCH3 WCH4

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgtaatacgtgcttcagtcataattgccagaggacaaattgttctctc
 ccagctcagcaatcctgtctgcacatccaggggagaaggtcacatgacttgcagggccagctcaagtgttaagtacatgcactgg
 25 taccagcagaagccagatcctccccaaacctggatttatgccccatccaacctggcttctggagtcacctgctcgttcagtggca
 gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttaaac
 ccaccacgttcgggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
 tctcaggcttatctacagcagtctggggctgagctgtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacatt
 taccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatgggtgatactt
 30 cctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagtcagcagcctgaca
 tctgaagactctgcggtctatttctgtgaagagtgggtgactatagtaactcttactggctacttcgatgtctggggcacagggaccacg
 gtcaccgtctctctgacacgttcgacctgtcaacatcactgagccacacttgaggctactccattcactcctgcgaccccaatgcattcc
 actccaccatccagctgtactgcttcatttatggccacatcctaaatgatgtctctgtcagctggctaattggacgatcgggagataactg
 atacacttgcaaaactgttctaataaggaggaaaggcaaaactagcctctacctgcagtaaaactcaacatcactgagcagcaatggat
 35 gtctgaaagcaccttcacactgcaaggtcacctcccaaggcgtagactatttgccacacactcgagatgccagatcatgagccacg
 gggtgtgattacactacctgatccaccagccccctggacctgtatcaaaacgggtgctcccaagcttacctgtctggtggtggacctg
 gaaagcgagaagaatgtcaatgtgacgtggaaccaagagaagaagacttcagctcagcatccagtggtactaagaccacaaa
 taacgccacaactagtatcacctccatcctgctgtagtggcaaggactggattgaaggctacggctatcagtgcatagtggaccac
 cctgattttcccaagccattgtgcttccatcaccaagacccagggcagcgctcagccccgaggtatatgtgttccaccaccag
 40 aggaggagagcgaggacaaacgcacactcactgtttgatccagaacttcttccctgaggatatctctgtgcagtggctgggggatg
 gcaaaactgatcacaacagccagcacagtaccacaacacctgaaatccaatggctccaatcaaggcttcttcatcttcagtcgcta
 gaggtcgaagacactctggacacagagaaaacagttcacctgccaagtatccatgaggcacttcagaaaccaggaaactgg
 agaaaaacaataccacaagccttgtaaaccttccctccgtccatcctagtaatactagag

45 Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
 sgshtsysltisrveaadaatyccqqsfnpptfgagtkleldggsgggsgggssqaylqqsgaesvrpgasvkmcks
 gytftsynmhwwkqtrqglewigaiypngdtsynqkfkgkatltvdkssstaymqllsitsedsavyfcarvvysnsywy
 fdvwtgtttvtvssdhvrpvniteptlellhsscdpnafhstiqlycfiyghilndvsvswlmdreitdtlaqtvlkeegklats
 5 klniteqqwmsestftckvtsqgvdyahtrrcpdheprgvitylippspldlyqngapklclvvdlesekvnvtnwnekkts
 vsasqwytkhhnnattsitsilpvvakdwiegygyqicvdhpdfpkpivrsitktpgqrsapevyvfppeeedsdkrtltcliq
 ffpedisvqwlgdgklisnshsttllksnsgnqgffisrlevaktlwtqrkqftcqvihcalqprklektistslgnslrps

17. 2H7 scFv VH L11S hIgA WH WCH2 T4CH3

10 Nucleotide sequence:

aagcttgccgcatggaatttcaagtgcagatttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
 gtgggtctgggaccttactctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 15 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
 tctcaggcttatctacagcagctctggggctgagctctgtaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacatt
 taccagttacaatatgcactgggtaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatactt
 cctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgaca
 tctgaagactctgcggtctatttctgtcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaaccag
 20 gtcaccgtctctctgcatcagccagttccctcaactccactacccatctccctcaactccactacccatctccctcatgctgccacc
 cccgactgtcactgcaccgaccggcctcgaggacctgctcttaggttcagaagcagatcctcacgtgcacactgaccggcctgaga
 gatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgtgttaaggaccacctgaccgtgacctctgtggctgc
 tacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttacttgactgctgcctaccccgagtc
 aagaccccgctaaccgccacctctcaaaatccggaacacattccggccgaggtccacctgctgccgccgctcggaggagc
 25 tggcctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggctgcaggggtca
 caggagctgccccgcgagaagtacgtgacttgggcatccggcgaggagccagccagggcaccaccaccttcgctgtgaccagc
 atactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggggcacgaggcctgccgtggccttca
 cacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcagggagggtgactgataatctaga

30 Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
 sgshtsysltisrveaadaatyccqqsfnpptfgagtkleldggsgggsgggssqaylqqsgaesvrpgasvkmcks
 gytftsynmhwwkqtrqglewigaiypngdtsynqkfkgkatltvdkssstaymqllsitsedsavyfcarvvysnsywy
 fdvwtgtttvtvssdqpvpstptpsptpspcchprlsrlhpaledlllgseailtctltglrdasgvtftwtpssgksavqgp
 35 pdrdlcgcysvssvlpgaepwnhgtftctaaypesktplatlkskngtfrpevhllpppseelalnelvtltclargfspkdv
 lvrwlqgsqelprekyltwasrqepsqgtttfavtsilrvaedwkkgdtfscmvghcalplafqtidrlagkpthvnnsvvmaev
 d

18. 2H7 scFv VH L11S mIgA WH WCH2 T4 CH3

40 Nucleotide sequence:

aagcttgccgcatggaatttcaagtgcagatttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
 gtgggtctgggaccttactctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
 45 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
 tctcaggcttatctacagcagctctggggctgagctctgtaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacatt

taccagttacaatatgactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatactt
cctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgaca
tctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccacg
gtcaccgtctcttctgatcacatctgttctcctactactcctcctccaccttctgccagcccagcctgtcactgcagcggccagctc
5 ttgaggacctgctcctgggttcagatgccagcatcacatgtactctgaatggcctgagagatcctgaggagctgtcttcacctggga
gccctccactgggaaggatgcagtgcagaagaaagctgtgcagaattcctgcggctgctacagtgtgtccagcgtcctgcctggctg
tgctgagcgtggaacagtggcgcacattcaagtgcacagttaccatcctgagctgtacaccttaactggcacaattgccaaagtc
acagtgaacaccttcccacccaggtccacctgctaccgccgccgtcggaggagctggccctgaatgagctcgtgtccctgacatg
cctgggtgcgagctttcaaccctaaagaagtgtgtgcatggctgcatggaaatgaggagctgtccccagaaagctacctagtgtt
10 gagccctaaaggagccaggcgagggagccaccacctacctggtgacaagcgtgttgcgtgtatcagctgaaatctggaacagg
gtgaccagtactcctgcatggtgggccacgaggccttggccatgaacttcaccagaagaccatcgaccgtctgtcgggtaaaccaca
ccaatgtcagcgtgtctgtgatcatgtcagaggagattgataatctagat

Amino acid sequence:

15 mdfqvqifslisasviiargqivlsqspailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveadaatyycqwsfnpptfagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsymbhwwkqtrpqglewigaiypngdtsynqfkfkatltvdksstaymqlssltsedsavyfcarvvyysnsywy
fdvwtgtttvssdhicspptppppscqpslslqrpaledlllgdsasitctlnlrdpegavftwepstgkdavqkkavqnsccg
cysvssvlpgcaerwnsgasfkctvthpesdltltgiakvtvntfppqvhlpppseelalnelvslclvrafnpkevlvrwlhgn
20 eelspesylvfeplkepegattylvtsvlrsvaeiwwkqgdqyscmvghealpmnftqktidrlsgkptnsvsvimsegd

A. mIgA WCH2 T4CH3

Nucleotide sequence:

Gttgttgatcacatctgttctcctactactcctcctccaccttctgccagcccagcctgtcactgcagcggccagctcttgaggac
25 ctgctcctgggttcagatgccagcatcacatgtactctgaatggcctgagagatcctgaggagctgtcttcacctgggagccctcca
ctgggaaggatgcagtgcagaagaaagctgtgcagaattcctgcggctgctacagtgtgtccagcgtcctgcctggctgtgctgagc
gctggaacagtggcgcacattcaagtgcacagttaccatcctgagctgtacaccttaactggcacaattgccaaagtcacagtga
caccttcccacccaggtccacctgctaccgccgccgtcggaggagctggccctgaatgagctcgtgtccctgacatgcctggtgc
gagctttcaaccctaaagaagtgtgtgcatggctgcatggaaatgaggagctgtccccagaaagctacctagtgtttgagccct
30 aaaggagccaggcgagggagccaccacctacctggtgacaagcgtgttgcgtgtatcagctgaaatctggaacagggtgaccag
tactcctgcatggtgggccacgaggccttggccatgaacttcaccagaagaccatcgaccgtctgtcgggtaaaccaccaatgtc
agcgtgtctgtgatcatgtcagaggagattgataatctagat

Amino acid sequence:

35 dhicspptppppscqpslslqrpaledlllgdsasitctlnlrdpegavftwepstgkdavqkkavqnsccgcysvssvlpgcaer
wnsgasfkctvthpesdltltgiakvtvntfppqvhlpppseelalnelvslclvrafnpkevlvrwlhgneelspesylvfeplk
epgegattylvtsvlrsvaeiwwkqgdqyscmvghealpmnftqktidrlsgkptnsvsvimsegd

20. K322S CH2 region

Nucleotide sequence:

40 cctgaactcctggggggaccgtcagttctcttccccccaaaacccaaggacaccctcatgatctccggaccctgagggtcacat
gcgtggtgggtggacgtgagccacgaagacctgaggtcaagttaactggtagcgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtgtcagcgtcctaccgtcctgcaccaggactggtgaatggcaa
ggagtacaagtgtcgtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaa

45 Amino acid sequence:

pellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwln
gkeykcsvsnk alpapiektiskak

21. K322S CH2 WCH3

5 Nucleotide sequence:

cctgaactcctggggggaccgtcagttctcttcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacat
gcgtgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaa
ggagtacaagtgtcgtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgaga
10 accacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtaaaggcttctatc
ccagcgacatcgccgtggagtgaggagcaatggcgagccggagaaactacaagaccacgcctcccgtgctggactccgac
ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

15 Amino acid sequence:

Pellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwln
gkeykcsvsnk alpapiektiskakgqprepvytlppsrdeltknqvsltclvkgyfypsdiavewesngqpennykttppvl
dsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispk

20 1. K322L CH2 WCH3

Nucleotide sequence:

tgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggaccgtcagttctctc
tcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtgggtgggtgacgtgagccacgaagacc
tgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcac
25 gtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcctggctccaacaaagccct
cccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccggga
tgagctgaccaagaaccaggtcagcctgacctgcctggctcaaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg
ggcagccggagaaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggac
aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc
30 cctgtctccgggtaaatgatctaga

Amino acid sequence:

Dqepkssdkhtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeq
ynstyrvvsvltvlhqdwlngkeykclvsnk alpapiektiskakgqprepvytlppsrdeltknqvsltclvkgyfypsdiave
35 wesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispk

22. 2H7 scFv VHL11S hIgG1 (SSS-S)H K322SCH2 WCH3

40 Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatcagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctcagcaatctgtctgcattctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgttcagtgcca
gtgggtctgggaccttctctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtgagggtttaac
45 ccaccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtgggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagtcggtagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat

ttaccagttacaatatgcactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
 tcctacaatcagaagttaagggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac
 atctgaagactctgcggcttatttctgtgcaagagtgggtgactatagtaactttacttggtacttcgatgtctggggcacaggaccac
 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga
 5 ccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtga
 gccacgaagaccctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgtcggctt
 ccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgc
 ccccatccccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctatcccagcgacatcgccgtggag
 10 tgggagagcaatgggcagccggagacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaa
 gctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatagaggtctgcacaaccactacacgc
 agaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

15 mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
 sgsqtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
 gyftsynmhvkwqtprrglewigaiypngdtsynqkfkglkatltvdksstaymqlssltsedsavyfcarvvysnsywy
 fdvwtggtvtvssdqepkssdkthtsppsapellggpsvflfppkpdltlmisrtpetcvvvdvshedpevkfnwyvdgv
 evhnaktkpreeqynstyrvsvltvlhqdwlngkeykcsvsnkalpapiektiskakgqprepqvylppsrdeltknqvsltc
 20 lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhtqkslsispkg

23. 2H7 scFv VHL11S hIgG1 (SSS-S)H K322LCH2 WCH3

Nucleotide sequence:

25 aagcttgccgcatggtatttcaagtgcagattttagcttctgtaatacgtgcttcagtcataattgccagaggacaaattgttctctc
 ccagctccagcaatcctgtctgcacatccaggggagaaggtcacatgacttgaggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggtacatctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtggca
 gtgggtctgggaccttactcttcacaaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggtttaac
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagc
 30 tctcaggcttatctacagcagctctggggtgagtcgggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat
 ttaccagttacaatatgcactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
 tcctacaatcagaagttaagggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac
 atctgaagactctgcggcttatttctgtgcaagagtgggtgactatagtaactttacttggtacttcgatgtctggggcacaggaccac
 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga
 35 ccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtga
 gccacgaagaccctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcctggtct
 ccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgc
 ccccatccccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctatcccagcgacatcgccgtggag
 40 tgggagagcaatgggcagccggagacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaa
 gctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatagaggtctgcacaaccactacacgc
 agaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

45 mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
 sgsqtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas

gytftsynmhvwkqtrpqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltsedsavyfcarvvyysnsywy
fdvwtgtgtvtvssdqepkssdkthtsppssapellggpsvflfppkpkdtlmsirtpevtcvvvdvshedpevkfnwyvdgv
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykclvsnkalspapiektiskakgqprepvytlppsrdeltknqvsltl
vkgfypsdiavewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhhealhnhytqkslsispkg

5

24. 2H7 scFv VHL11S hIgG1 (CSS-S)H K322SCH2 WCH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttcagggccagctcaagtgaagtacatgcactgg
10 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
gtgggtctgggaccttctactcttcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcgggtggtggtatctggaggaggtgggagc
tctcaggttatctacagcagctctggggctgagtcggtgagggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
15 tctacaatcagaagtcaagggaagggcacactgactgtagacaaaatctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaactttactggtacttcgatgtctggggcacaggggaccac
ggtcaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga
ccgtcagttctcttctccccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcggtggaggtgcataatgccaagacaaagccgcgaggagc
20 agtacaacagcacgtaccgtgtgtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgtcgggtct
ccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgc
ccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctatcccagcgacatcgccgtggag
tgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtggactccgacggctccttcttctctacagcaa
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggctctgcacaaccactacacgc
25 agaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveadaatyycqwsfnpptfagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
30 gytftsynmhvwkqtrpqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltsedsavyfcarvvyysnsywy
fdvwtgtgtvtvssdqepksdkthtsppssapellggpsvflfppkpkdtlmsirtpevtcvvvdvshedpevkfnwyvdgv
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykcsvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltc
lvkgfypsdiavewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhhealhnhytqkslsispkg

35 25. P331S CH2

Nucleotide sequence:

cctgaactcctggggggaccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacat
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcggtggaggtgcataatgccaagac
aaagccgcgggaggagcagtagaacagcacgtaccgtgtgtcagcgtcctaccgtcctgcaccaggactggctgaatggcaa
40 ggagtacaagtgaaggtctccaacaagccctccagcctccatcgagaaaacaatctccaaagccaaa

Amino acid sequence

Pellggpsvflfppkpkdtlmsirtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlng
45 keykckvsnkalpasiektiskak

26. P331S CH2 WCH3

Nucleotide sequence:

cctgaactcctggggggaccgtcagttcttcttcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacat
gcgtgggtggacgtgagccacaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatccaagac
5 aaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaa
ggagtacaagtcaaggtctccaacaagccctcccagcctccatcgagaaaacaatctccaaagccaaagggcagccccgaga
accacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctatc
ccagcgacatcgccgtggagtgaggagcaatgggcagccggagacaactacaagaccagcctcccgtgctggactccgac
ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
10 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

15 Amino acid sequence

pellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdw
ngkeykckvsnkalpasiektiskakgqprepvytlppsrdektknqvsitclvkgfypsdiavewesngqpennykttppvl
dsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

20 27. 2H7 scFv VH L11S (SSS-S)H P331S CH2 WCH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcacatccaggggagaaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctctgcttcagtgga
25 gtgggtctgggaccttctactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggtgagtcggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat
ttaccagttacaatatgactgggtaaagcagacacctagacaggcctggatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagtcaagggaagccacactgactgtagacaatctccagcacagcctacatgcagctcagcagcctgac
30 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttctactggtacttcgatgtctggggcacaggaccac
ggtcaccgtctctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga
ccgtcagttcttcttcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtgggtggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaagggtc
35 tccaacaaagccctcccagcctccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgc
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctatcccagcgacatcgccgtggag
tgggagagcaatgggcagccggagaacaactacaagaccagcctcccgtgctggactccgacggctccttcttctctacagcaa
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

40

Amino acid sequence

mdfvqqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsynmhwwkqtrpqglewigaiypngdtsynqkfkkgkatltvdksstaymqlssltedsavyfcarvvyysnsywy
45 fdvwtgtttvssdqepkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv

evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpasiektiskakgqprepvytlppsrdeltknqvsltc
lvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslsispkg

5 **28. 2H7 scFv VH L11S (CSS-S)H P331S CH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctcagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccagatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca
10 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtttaac
ccaccacggttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtgatctggaggaggtgggagc
tctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagtcaagggaagccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
15 atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaggagcccaaatcttgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga
ccgtcagcttctcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcagctaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc
20 tccaacaaagccctcccagcctccatcgagaaaacaatcctcaaaagccaaagggcagccccgagaaccacaggtgtacaccctgc
ccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctggctcaaaggcttctatcccagcgacatcgcctggag
tgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaa
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

25

Amino acid sequence

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg
sgsgtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsggggssqaylqqsgaesvrpgasvkmsckas
gytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvyysnsywy
30 fdvwgtgtvtvssdqepkscdkthtspssapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpasiektiskakgqprepvytlppsrdeltknqvsltc
lvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslsispkg

35 **29. T256N CH2 region**

Nucleotide sequence:

Cctgaactcctggggggaccgtcagcttctcttccccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcaca
tgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaga
caaagccgcgggaggagcagtacaacagcagctaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa
ggagtacaagtgaaggctctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaa

40

Amino acid sequence

pellggpsvflfppkpkdtlmisrnpvtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwl
lngkeykckvsnkalpapietiskak

45 **30. T256N CH2 WCH3**

Nucleotide sequence:

cctgaactcctggggggaccgtcagttcttcttcccccaaaacccaaggacaccctcatgatctcccgaaccctgaggtcacat
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggagggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaa
ggagtacaagtgaaggtctccaacaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgaga
5 accacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgggtcaaaggcttctatc
ccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

10

Amino acid sequence

pellggpsvflfppkpkdtlmisrnpvctvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdw
lngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitclvkgyfypsdiavewesngqpennykttppv
15 ldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispk

31. 2H7 scFv VH L11S (SSS-S)H T256N CH2 WCH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtcagatttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc
20 ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacatgacttgaggccagctcaagtgaagtacatgcactgg
taccagcagaagccaggatcctccccaaaccctggatttatccccatccaacctggcttctggagctccctgctcgttcagtgga
gtgggtctgggacctctactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggaagttaac
ccaccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
25 tctcaggcttatctacagcagctctggggtgagtcggtagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga
30 ccgtcagttctcttcccccaaaacccaaggacaccctcatgatctcccgaaccctgaggtcacatgcgtggtggtggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggtgaatggcaaggagtacaagtgaaggtc
tccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgggtcaaaggcttctatccagcgacatcgccgtgga
35 gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgcatgaggctctgcacaaccactacacg
cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

mdfqvqifsfllisasviiargqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg
sgsgtsysltisrveadaatyycqqwsfnpptfgagtkleldggsgsgggsgggssqaylqqsgaesvrgasvkmckas
gytftsynmhwwkqtrpqrlewigaiypngdtsynqkfkgtatltvdksstaymqlssltedsavfyfcarvvyysnsywy
fdvwgtgtvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmisrnpvctvvvdvshedpevkfnwyvdgvev
evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitc
45 lvkgyfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispk

32. 2H7 scFv VH L11S (CSS-S)H T256N CH2 WCH3

Nucleotide sequence:

5 aagcttgccgcatggaatttcaagtgcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgaagttacatgcactgg
taccagcagaagccagatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
gtgggtctgggaccttctactctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
tctcaggcttatctacagcagcttggggctgagtcggtgagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
10 ttaccagttacaataatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagtcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacaggggaccac
ggtcaccgtctcttctgatcaggagccaaatcttgtacaaaactcacatccccaccgtctcagcacctgaactcctgggggga
ccgtcagcttctcttccccccaaaacccaaggacacctcatgatctccggaacctgaggtcacatgcgtgggtggagcgtga
15 gccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaaagccgaggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggctc
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctg
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca
20 agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg
cagaagagcctctccctgtctccggtaaatgatctaga

Amino acid sequence

25 mdfvqvifsfllisasviiargqivlsqspailsaspgekvtmtrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveaadaatyccqqsfnpptfgagtkleldggsgsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvyysnsywy
fdvwtgtttvssdqepkscdkthtsppssapellggpsvflfppkpkdtlmisnpevtcvvvdvshedpevkfnwyvdgv
evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvylppsrdeltnqvsltc
lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhhealnhhtqkslsispkg

33. RTPE/QNAK (255-258) CH2

Nucleotide sequence:

35 cctgaactcctggggggaccgtcagttctcttccccccaaaacccaaggacacctcatgatctcccagaacgctaaggctcacat
gcgtggtgggtggacgtgagccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa
ggagtacaagtgaaggctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaa

Amino acid sequence

40 pellggpsvflfppkpkdtlmisqnakvtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdw
lngkeykckvsnkalpapiektiskak

34. RTPE/QNAK (255-258)CH2 WCH3

Nucleotide sequence:

45 cctgaactcctggggggaccgtcagttctcttccccccaaaacccaaggacacctcatgatctcccagaacgctaaggctcacat
gcgtggtgggtggacgtgagccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa

ggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgaga
 accacaggtgtacaccctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgggtcaaaggcttctatc
 ccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgctcccgtgctggactccgac
 ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgcatgag
 5 gctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaatgatctaga

Amino acid sequence

pellggpsvflfppkpkdtlmsiqnakvtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqd
 lngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltnqvslclvkgyfypsdiavewesngqpennyktpvp
 10 ldsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslspsgk

35. 2H7 scFv VH L11S (SSS-S)H RTPE/QNAK (255-258)CH2 WCH3

Nucleotide sequence:

15 aagcttgccgcatggtatttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
 gtgggtctgggaccttctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc
 20 tctcaggcttatctacagcagctctggggtgagtcggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
 tctacaatcagaagttaagggaagccacactgactgtagacaatactccagcacagcctacatgcagctcagcagcctgac
 atctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
 ggtcaccgtctctctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga
 25 ccgtcagttctctcttccccccaaaacccaaggacacccctcatgatctcccagaacgtaaggtcacatgcgtgggtggagtgga
 gccacgaagaccctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagc
 agtacaacagcacgtaccgtgtgtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc
 tccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaaggcagccccgagaaccacaggtgtacaccctg
 cccccatccgggatgagctgaccaagaaccaggtcagcctgacctgctgggtcaaaggcttctatccagcgacatcgccgtgga
 30 gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtggactccgacggctccttctctctacagca
 agctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgcatgaggctctgcacaaccactacacg
 cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

35 mdfqvqifsflisasviiargqivlsqspailsaspgekvmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
 sgsqtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
 gytftsynmhwwkqtrqglewigaiypngdtsynqfkkgkatltvdkssstaymqlssltedsavyfcarvvysnsywy
 fdvwtgtttvtssdqepkssdkthtspssapellggpsvflfppkpkdtlmsiqnakvtcvvvdvshedpevkfnwyvdg
 evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltnqvsltc
 40 lvkgyfypsdiavewesngqpennyktpvpldsdsfflyskltvdksrwqqgnvfscsvmhealnhytqkslspsgk

36. 2H7 scFv VH L11S (CSS-S)H RTPE/QNAK (255-258)CH2 WCH3

Nucleotide sequence:

45 aagcttgccgcatggtatttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcactgg
 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga

gtgggtctgggacctcttactctctcacaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtgagggttttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagtcggtgagggcctggggcctcagtgaagatgctcgaaggcttctggctacacat
ttaccagttacaatatgcactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
5 tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga
ccgtcagttctctcttcccccaaaacccaaggacaccctcatgatctcccagaacgctaaggtcacatgcgtgggtgggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagc
10 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctg
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaactcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg
15 cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

mdfqvqifsfllisasviiargqivlsqspailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveadaatyycqqsfnpptfgagtkleldggsgggsgggssqaylqqsgaesvrpgasvkmsckas
20 gytftsymbhwwkqtpqrqglewigaiypngdtsynqkfkkgkatltvdkssstaymqllsstsedsavyfcarvvysnsywy
fdvwtgttvtvssdqepkscdkthtsppssapellggpsvflfppkpkdtlmsqnakvtcvvvdvshedpevkfnwyvdg
vevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrldeltnqvslt
clvkgyfypsdiavewesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslspgk

25 36. K290Q CH2 region

Nucleotide sequence:

cctgaactcctggggggaccgtcagttctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacat
gcgtgggtgggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac
acagccgaggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa
30 ggagtacaagtgaagggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaa

Amino acid sequence:

pellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktqpreeqynstyrvsvltvlhqdwl
ngkeykckvsnkalpapiektiskak

35

37. K290Q CH2 WCH3

Nucleotide sequence:

Cctgaactcctggggggaccgtcagttctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcaca
tgcgtgggtgggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaaga
40 cacagccgaggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa
ggagtacaagtgaagggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgaga
accacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatc
ccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
45 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

pellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktqpreeqynstyrvsvltvlhqdwl
ngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitclvkgyfypsdiavewesngqpennykttppvl
dsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

5

38. 2H7 scFv VH L11S (SSS-S)H K290Q CH2 WCH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg
10 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggtacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
15 tctacaatcagaagtcaagggaaggccacctgactgtagacaaaatctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga
ccgtcagcttctcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacacagccgcgggaggagc
20 agtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc
tccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgttgactccgacggctccttctctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaactcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg
25 cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdfvqqifsfllisasviiargqivlsqspailsaspgekvtmtrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveadaatyycqwsfnpptfagtkleldggsgggsgggssqaylqqsgaesvrpgasvkmsckas
30 gytftsynmhvkwqtpqrqglewigaiypngdtsynqfkkgkatltvdksstaymqlssltedsavyfcarvvvysnsywy
fdvwtggtvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv
evhnaktqpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitc
lvkgyfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

39. 2H7 scFv VH L11S (CSS-S)H K290Q CH2 WCH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg
40 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga
gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggtacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagtcaagggaaggccacctgactgtagacaaaatctccagcacagcctacatgcagctcagcagcctgac
45 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga

ccgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacacagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggtgaatggcaaggagtacaagtgaaggtc
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaaggcagccccgagaaccacaggtgtacaccctg
5 ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
cagaagagcctctccctgtctccgggtaaatgatctaga

10 Amino acid sequence:
mdfqvqifsflisasviiargqivlsqspailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsymbhwwkqtrprglewigaiypngdtsynqkfkgkatltvdkssstaymqlssltedsavyfcarvvysnsywy
fdvwtgtttvtssdqepkscdkthtsppssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv
15 evhnaktqpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsltc
lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhtqkslspsgk

40. A339PCH2

Nucleotide sequence:

20 cctgaactcctggggggaccgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacat
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggtgaatggcaa
ggagtacaagtgcagggtctccaacaaagccctcccagccccatcgagaaaacaatctccaacccaaa

25 Amino acid sequence:
pellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktqpreeqynstyrvsvltvlhqdw
lngkeykckvsnkalpapiektiskpk

30 41. A339P CH2 WCH3

Nucleotide sequence:

cctgaactcctggggggaccgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacat
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggtgaatggcaa
35 ggagtacaagtgcagggtctccaacaaagccctcccagccccatcgagaaaacaatctccaacccaaagggcagccccgaga
accacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctggtgtaaaaggcttctatc
ccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

40 Amino acid sequence:
pellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktqpreeqynstyrvsvltvlhqdw
lngkeykckvsnkalpapiektiskpkgpprepqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennyktpv
45 dsdgsfflyskltvdksrwqqgnvfscsvmhealnhhtqkslspsgk

42. 2H7 scFv VHL11S (SSS-S)H A339P CH2 WCH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctctgctcgttcagtggca
5 gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtgatctggaggaggtgggagc
tctcaggcttatctacagcagcttggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
10 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggggaccac
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga
ccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgggtggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtgtgcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc
15 tccaacaaagccctcccagccccatcgagaaaacaatctcaaaccctaaagggcagccccgagaaccacaggtgtacacctgc
ccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctggcctaaaggcttctatcccagcgacatcgccgtggag
tgggagagcaatggcgagccgggagaacaactacaagaccacgcctccgtgctggactccgacggctccttcttctctacagcaa
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaacctacacgc
agaagagcctctcctgtctccgggtaaatgatctaga
20

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgkvtmtrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg
sgsgtsysltisrveadaatyycqqlwfnppftfagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsymbhwvkqprqglewigaiypngdtsynqfkfkgkatltvdksstaymqlssltedsavyfcarvvysnsywy
25 fdvwtgtttvssdqepkssdkthtspssapellggpsvflfpkpkdltlmisrtpetcvvvdvshedpevkfnwyvdgv
evhnaktkpreeqynstyrvvsvltvlhqdwlngkeyckvsnkalpapiektiskpkqpprepqvytlppsrldtknqvsltc
lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispkg

30 43. 2H7 scFv VHL11S (CSS-S)H A339P CH2 WCH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctctgctcgttcagtggca
35 gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtgatctggaggaggtgggagc
tctcaggcttatctacagcagcttggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
40 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggggaccac
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga
ccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgggtggacgtga
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtgtgcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc
45 tccaacaaagccctcccagccccatcgagaaaacaatctcaaaccctaaagggcagccccgagaaccacaggtgtacacctgc
ccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctggcctaaaggcttctatcccagcgacatcgccgtggag

tgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaa
gctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

5 Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgkevmtcrasssvsymhwyqqkpgsspkpwiypslnlasgvparfsg
sgsgtsysltisrveadaatyycqqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsynmhwwkqtrpqrqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvysnsywy
fdvwgtgtvtvssdqepkscdkthtspssapellggpsvflfppkpkdtlmisrtpetvcvvvdvshedpevkfnwyvdgv
10 evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskpkgpprepqvylppsrdeltknqvsltc
lvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslsispkg

44. G28-1VH

15 Nucleotide sequence:

gcgggtccagctgcagcagcttggaacctgagctggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttactcattca
ctggctacaatatgaactgggtgaagcagaataatggaagagccttgagtggattggaaatattgatccttattatggtggtactacct
acaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctg
aggactctgcagcttattactgtgcaagatcggtcggccctatggactactgggggtcaaggaacctcagtcaccgtctctctgatcag

20

Amino acid sequence:

avqlqqsgpelekp gasvkisckasgysftgynmnwvkqnnngkslewnidpyyggtynrkfkkgkatltvdkssstaymq
lksltedsavyycarsvgpmdywgqgtsvtvssdq

25 45. G28-1VL

Nucleotide sequence:

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca
gtctccagcctccctatctgcattctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggcttggtat
cagcagaaacagggaaaatctcctcagctcctggctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg
30 gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatcattccgataatccgtg
gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcggcggtggtgggtcgggtggcgggcggatcgtca

30

Amino acid sequence:

mvstaqlflgllllwltggrcdiqmtqspaslsasvgetvtitcrtsenvysylawyqqkqkspqllvsfaktlaegvpsrfsghs
35 gtqfslkisslqpedsqsyfcqhhsdpwtfgggteleikggggsgggsgggss

46. G28-1 scFv

Nucleotide sequence:

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca
gtctccagcctccctatctgcattctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggcttggtat
cagcagaaacagggaaaatctcctcagctcctggctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg
gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatcattccgataatccgtg
gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcggcggtggtgggtcgggtggcgggcggatcgtcag
45 cgggtccagctgcagcagcttggaacctgagctggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttactcattcact
ggctacaatatgaactgggtgaagcagaataatggaagagccttgagtggattggaatatgatccttattatggtggtactacctac

aaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctga
ggactctgcagctattactgtgcaagatcggcggccctatggactactggggtcaaggaacctcagtcaccgtctcttctgatcag

Amino acid sequence:

5 mvstaqflglillwtggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsghsgs
gtqfslkisslqpedsgsyfcqhhsdnptwtfgggteleikggggsgggsgggssavqlqqsgpelekp gasvkiskcasgys
ftgynmnwvkqnnkslewnidpyyggtynrkfk gkatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg
tsvtvssdq

10

47. G28-1 VHL11S

Nucleotide sequence:

gcgggtccagctgcagcagctggacctgagtcggaaaagcctggcgcttcagtgagatttcctgcaaggcttctggttactcattca
ctggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtgattggaaatattgatccttattatggtggtactacct
15 acaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctga
aggactctgcagctattactgtgcaagatcggcggccctatggactactggggtcaaggaacctcagtcaccgtctcttctgatcag

Amino acid sequence:

20 avqlqqsgpesekpgasvkiskcasgysftgynmnwvkqnnkslewnidpyyggtynrkfk gkatltvdkssstaym
qlksltsedsavyycarsvgpmdywgqgtsvtvssdq

48. G28-1 VHL11S scFv

Nucleotide sequence:

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacagggtggcagatgtgacatccagatgactca
gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttgcttggtat
cagcagaaaacagggaaaatctctcagctcctgggtctcttttgcaaaaaccttagcagaaggtgtgccatcaagggtcagtggcagtg
gatcaggcacacagtttctctgaagatcagcagcctgcagcctgaagattctggaagttattctgtcaacatcattccgataatccgtg
30 gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcggcggtggtgggtcgggtggcggcggatcgtcag
cgggtccagctgcagcagcttgacctgagtcggaaaagcctggcgcttcagtgagatttcctgcaaggcttctggttactcattcact
ggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtgattggaaatattgatccttattatggtggtactacctac
aaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctga
ggactctgcagctattactgtgcaagatcggcggccctatggactactggggtcaaggaacctcagtcaccgtctcttctgatcag
35

Amino acid sequence:

mvstaqflglillwtggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsghsgs
gtqfslkisslqpedsgsyfcqhhsdnptwtfgggteleikggggsgggsgggssavqlqqsgpesekpgasvkiskcasgys
ftgynmnwvkqnnkslewnidpyyggtynrkfk gkatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg
40 tsvtvssdq

49. G28-1 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence:

45 aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacagggtggcagatgtgacatccagatgactca
gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttgcttggtat

cagcagaaacagggaaaatctcctcagctcctggtctcttttgcaaaaccttagcagaaggtgtgcatcaaggttcagtggcagtg
 gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatattccgataatccgtg
 gacgttcggtggagggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtggcgccggatcgtcag
 cgggtccagctgcagcagctctggacctgagctggaaaagcctggcgcttcagtgaaagtattcctgcaaggcttctggttactattcact
 5 gggtacaatatgaactgggtgaagcagaataatggaaagagccttgagtggttgaaatattgatccttattatggtggtactacctac
 aaccgggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagcttgacatctga
 ggactctgcagctctattactgtgcaagatcggtcggccctatggactactgggtcaaggaacctcagtcaccgtctcttctgatcatg
 atcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggaccgtcagcttctcttct
 ccccaaaaaccaaggacacctcatgatctccggaccttgaggtcacatgcgtgggtgggtggacgtgagccacgaagacctg
 10 aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgt
 accgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtctccaacaaagccctc
 ccagcccccacagagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggtgat
 gagctgaccaagaaccaggtcagcctgacctgcctggtcaaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg
 ggcagccgggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttcttctctacagcaagctcaccgtggac
 15 aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc
 cctgtctccgggtaaatgatctaga

Amino acid sequence:

mvstaqlglilllwtggrediqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfssgsgs
 20 gtqfslkisslqpedsgsyfcqhhsdnptwfgggteleikggggsgggsgggssavqlqsgpelekpgasvkiskcasgys
 ftgynmnwvkqnnkslewignidpyyggtytnrkfkkatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg
 tsvtvssdhdepkssdkthtspssapellggspsvflfppkpkdtlmisrtpcvvvdvshedpevkfnwyvdgvevhna
 ktkpreeqynstyrvvsvltvlhqdwlnkeykckvsnkalpapiektiskakgpprepqvylppsrdeltknqvsitclvkqf
 ypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk
 25

50. G28-1 scFv IgAW H IgG1WCH2 WCH3

Nucleotide sequence:

aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctggttacaggtggcagatgtgacatccagatgactca
 30 gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggttggtat
 cagcagaaacagggaaaatctcctcagctcctggtctcttttgcaaaaccttagcagaaggtgtgcatcaaggttcagtggcagtg
 gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatattccgataatccgtg
 gacgttcggtggagggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtggcgccggatcgtcag
 cgggtccagctgcagcagctctggacctgagctggaaaagcctggcgcttcagtgaaagtattcctgcaaggcttctggttactattcact
 35 gggtacaatatgaactgggtgaagcagaataatggaaagagccttgagtggttgaaatattgatccttattatggtggtactacctac
 aaccgggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagcttgacatctga
 ggactctgcagctctattactgtgcaagatcggtcggccctatggactactgggtcaaggaacctcagtcaccgtctcttctgatcagc
 cagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgcgcacctgaactcctggggggaccgtca
 gtcttctcttcccccaaaaaccaaggacacctcatgatctccggaccttgaggtcacatgcgtgggtgggtggacgtgagccac
 40 gaagaccttgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtac
 aacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtctccaa
 caaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccc
 atccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaaggcttctatcccagcgacatcgccgtggagtggg
 agagcaatgggcagccggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttcttctctacagcaagctc
 45 accgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcaga
 agagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsqsgs
gtqfslkisslqpedsgsyfcqhhsdnpwtfgggteleikggggsgggsgggssavqlqsgpelekpgasvkiskasgys
5 ftgynmnwvkqnnkslewnidpyyggtynrkfkkgkatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg
tsvtvssdqvpstptpspstpptspscapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvev
hnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvylppsrdeitknqvsitclv
kgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispkg

10 51. **G28-1 scFv VHL11S (SSS-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca
gtctccagctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggttggtat
cagcagaaacagggaaaatctcctcagctcctgtctcttttgcacaaaccttagcagaaggtgtccatcaaggttcagtggcagtg
15 gatcaggcacacagttttcttgaagatcagcagcctgcagcctgaagattctggaagtattttctgtaacatcattccgataatccgtg
gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtggcggcggtatcgtcag
cgggtccagctgcagcagcttgacgtgagtcggaaaagcctggcgcttcagtgaaagttcctgcaaggcttctggttactcattcact
gggtacaatatgaactgggtgaagcagaataatggaaagagccttgagtggttggaataatgatccttattatggtgtactacctac
aaccgggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctgacatctga
20 ggactctgcagctattactgtgcaagatcggcggcctatggactactgggtcaaggaacctcagtcaccgtctcttctgacagg
agcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggaccgtcagcttctcttcccccc
aaaaccaaggaacacctcatgatctcccgaccctgaggtcacatcggtgggtgggtggacgtgagccacgaagacctgaggtc
aagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaacagcacgtaccgt
gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagc
25 ccccatcgagaaaacaatctccaaagcgaaggcagccccgagaaccacaggtgtacacctgccccatcccggtatgagct
gaccaagaaccaggtcagcctgacctgcctgggtcaaaggtcttatccagcgacatcgccgtggagtgaggagcaatgggcag
ccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagag
caggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtc
tccgggtaaatgatctaga

30

Amino acid sequence:

mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsqsgs
gtqfslkisslqpedsgsyfcqhhsdnpwtfgggteleikggggsgggsgggssavqlqsgpesekpgasvkiskasgys
35 ftgynmnwvkqnnkslewnidpyyggtynrkfkkgkatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg
tsvtvssdhdqepkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhna
ktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvylppsrdeitknqvsitclvkgf
ypsdiaavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispkg

40 52. **G28-1 scFv VHL11S (CSS-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca
gtctccagctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggttggtat
cagcagaaacagggaaaatctcctcagctcctgtctcttttgcacaaaccttagcagaaggtgtccatcaaggttcagtggcagtg
gatcaggcacacagttttcttgaagatcagcagcctgcagcctgaagattctggaagtattttctgtaacatcattccgataatccgtg
45 gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtggcggcggtatcgtcag
cgggtccagctgcagcagcttgacgtgagtcggaaaagcctggcgcttcagtgaaagttcctgcaaggcttctggttactcattcact

ggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaaatattgatccttattatgggtgtactacctac
 aaccgggaagtcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctgacatctga
 ggactctgcagtctattactgtgaagatcggtcgccctatggactactggggtcaaggaaacctcagtcaccgtctcttctgatcagg
 agcccaaatcttgtgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggaccgtcagttcttcttcccccc
 5 aaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtggacgtgagccacgaagacctgaggtc
 aagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgt
 gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagc
 ccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccgggatgagct
 gaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcag
 10 ccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggacaagag
 caggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgcagaagagcctctccctgtc
 tccggtaaatgatctaga

Amino acid sequence:

15 mvstaqlglillwtggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsghs
 gtqfslkisslqpedsgsyfcqhhsdnptwfgggtelkggggsgggsgggssavqlqsgpesekpgasvkiskcasgys
 ftgynmnwvkqnnkslewnidpyyggtynrkfkkatltvdksstaymqlksltsedsavyycarsvgpmdywgqg
 tsvtvssdqepksckdhtspssapellggsplfpkpkdltmisrtevtcvvdvshedpevkfnwyvdgvevhnakt
 kpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltclvkgyfyp
 20 sdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhnhytqkslsispkg

53. G28-1 scFv VH L11S (CSC-S)H WCH2 WCH3

Nucleotide sequence:

25 aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca
 gtctccagctccctatctgcacatgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggcttggtat
 cagcagaaacagggaaaatctctcagctcctggtctcttttgcaaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg
 gatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatattccgataatccgtg
 gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtggcggcgatcgtcag
 30 cggtcagctgcagcagcttgacctgagtcggaagacctggcgcttcagtgaaagattcctgcaaggcttctggttactcattcact
 ggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaaatattgatccttattatgggtgtactacctac
 aaccgggaagtcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctgacatctga
 ggactctgcagtctattactgtgaagatcggtcgccctatggactactggggtcaaggaaacctcagtcaccgtctcttctgatcagg
 agcccaaatcttgtgacaaaactcacacatctccaccgtgtcagcacctgaactcctgggtggaccgtcagttcttcttccccca
 35 aaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtggacgtgagccacgaagacctgaggtca
 agttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtg
 ggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagcc
 cccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccgggatgagctg
 accaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatgggcagc
 40 cggagaacaactacaagaccacgctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggacaagagc
 aggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgcagaagagcctctccctgtct
 ccgggtaaatgatctaga

Amino acid sequence:

45 mvstaqlglillwtggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsghs
 gtqfslkisslqpedsgsyfcqhhsdnptwfgggtelkggggsgggsgggssavqlqsgpesekpgasvkiskcasgys

ftgynmnwvkqnnkslewignidpyyggtynrkfkakatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg
tsvtvssdqepkscdkthtspcsapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnakt
kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsitclvkgyfyp
sdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

5

54. G28-1 scFv VH L11S (SSC-P)H WCH2 WCH3

Nucleotide sequence:

10 aagcttgccgccatgggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca
gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttgcttggtat
cagcagaaacagggaatatctcctcagctcctgtctcttttgcacaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg
gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatcattccgataatccgtg
gacgttcggtggaggcaccgaactggagatcaaaggtggcgtggctcgggcgggtgggtcgggtggcgccggtgacgtcag
cggctcagctgcagcagcttgacctgagtcggaaaagcctggcgcttcagtggaagatttctgcaaggcttctggttactcattcact
15 ggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggttggaatatgatccttattatggtgtactacactac
aaccggaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagcttgacatctga
ggactctgcagcttattactgtcaagatcggcggcctatggactactggggtcaaggaaacctcagtcaccgtctcttctgatcagg
agcccaaatcttctgacaaaactcacacatccccaccgtgccagcacctgaactcctggggggaccgtcagcttctcttcccccc
aaaaccaagacacccctcatgatctccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtc
20 aagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtagaacagcacgtaccgt
gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagc
ccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatcccggtgatgact
gaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccagcgacatcgccgtggagtgaggagcaatgggcag
ccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagag
25 caggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgcagaagagcctctccctgtc
tccgggtaaatgatctaga

Amino acid sequence:

30 mvstaqlglillwtggrcdiqmtqspaslsasvgetvtitrtstenvysylawyqqkqgkspqllvsfaktlaegvpsrfsqsgs
gtqfslkisslqpedsgsyfcqhhsdnptwtfgggteleikggggsgggsgggssavqlqqsgpesekpgasvkiskcasgys
ftgynmnwvkqnnkslewignidpyyggtynrkfkakatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg
tsvtvssdqepksscdkthtspcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnakt
kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsitclvkgyfyp
sdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

35

II. 54. HCTLA4 HIGG1 (SSS-S)H P238SCH2 WCH3

Nucleotide sequence:

40 atggcttgcccttggaattcagcggcacaaggctcagctgaacctggctgccaggacctggccctgcactctcctgtttttcttcttcat
ccctgtcttctgcaaaagcaatgcacgtggcccagcctgtgtgtgactggccagcagccaggcatcgccagcttctgtgtgagtat
gcattccaggcaaaagccactgaggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtcgggcaacct
acatgacggggaatgagttgaccttctagatgattccatctgcacgggcacctccagtggaatcaagtgaacctcactatccaagg
actgagggccatggacacgggactctacatctgcaaggtggagctcatgtaccaccgccatactacctgggcataggaacggaa
cccagatttatgtaattgatccagaaccgtgccagattctgatcaacccaaatcttctgacaaaactcacacatccccaccgtcctcag
cacctgaactcctggggggatcgtagtcttcttctccccccaaaaccaaggacacctcatgatctccggaccctgaggtcac
45 atcgtggtggtgacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag
acaaagccgcgggaggagcagtagaacagcacgtaccgtgtgtgcagctcctcaccgtcctgcaccaggactggctgaatggca

aggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgag
aaccacaggtgtacacctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctggcctgcaaggcttctat
cccagcgacatcgccgtggagtgaggagagcaatgggcagccggagaaactacaagaccacgcctcccgctgctggactccga
cggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
5 ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

Amino acid sequence:

maclgfqrhkaqlnlaartwpctllffllfipvfckamhvaqpavvlassrgiasfvceyaspgkatevrvtlrqadsqvtevaa
tymtgneltflddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyyidpepcpdsdpkssdkhts
10 ppssapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwvydgvvehnaktpreeqynstyrvvsvltvlh
qdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeitknqvsitclvkgfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslsispgk*

55. FC2-2 VL

15 Nucleotide sequence:

gttgtaagcttgccgccatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt
cacagctcccatctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtcagtcagagcctttatataatcacaatca
aaagaactacttgccctgggtaccagcagataaccagggcagctctcctaaactgctgatttactgggcatccactagggaaatctggggtc
cctgatcgcttcacaggcagtgatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg
20 tcagcaatattatcctatctctccacgttcggaggtggcaccagctggaaataaaagggtggcggtggctcgggcgggtgggtgggtc
gggtggcgggcgaggctcg

Amino acid sequence:

mdsqaqvlmllllwwsgtcddivmsqspsslavsvgekvsmckssqsllynhnqnknylawyqqipgqspklliywastres
25 gvpdrftgsgsgtdfilitisrvkaedlavyyccqyytypptfgggtkleikggggsgggsgggss

56. FC2-2VH

Nucleotide sequence:

Gggagctcgcaggtgcagttgaaggagtcaggacctggcctgggtggcgccctcacagagcctgtccatcacatgcaccgtctcag
30 ggttctcattaaccgtctatggtgtaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatggggatgatg
aagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagttttctaaaaatggacagtc
tacaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggctcaaggaaacctc
agtcaccgtctcctctgatcag

35 Amino acid sequence:

gssqvqlkesgpglvapsqslsitctvsgfsltygvnwvrpppgkglwdlwmigwdgstdynsalksrslskdsksqvflk
mdslqtdtdaryycardhygthyamdywgqtsvtvssdq

40 57. FC2-2scFv

Nucleotide sequence:

gttgtaagcttgccgccatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt
cacagctccatctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtcagtcagagcctttatataatcacaatca
aaagaactacttgccctgggtaccagcagataaccagggcagctctcctaaactgctgatttactgggcatccactagggaaatctggggtc
45 cctgatcgcttcacaggcagtgatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg
tcagcaatattatcctatctctccacgttcggaggtggcaccagctggaaataaaagggtggcggtggctcgggcgggtgggtgggtc

gggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggcctggcggccctcacagagcctgtccatcacatgc
accgtctcaggggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatg
gggtgatggaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagtttcttaaaa
atggacagtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtc
5 aaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:

mdsqaqvlmllllwwsgtcgdivmsqspsslavsvgekvsmckssqsllynhnqnknylawyqqipgqspklliywastres
gvpdrftgsgsgtdfltlisrvkaedlavyyccqyytypptfgggtkleikgggsgggsgggssqvqlkesgpglvapsql
10 sitctvsgfsltyvgvnnwvrppgkglwdlmiwgdgstdynsalksrslskdnksqvflkmdslqddtaryycardhygt
hyamdywgqgtsvtvssdq

58. FC2-2 VHL11S

15 Nucleotide sequence:

gggagctctcaggtgcagttgaaggagtcaggacctggctcggcggcgcctcacagagcctgtccatcacatgcaccgtctcagg
gttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatggggtgatgga
agcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagtttcttaaaaatggacagctc
20 acaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtcaaggaacctca
gtcaccgtctcctctgatcag

Amino acid sequence:

(gss)qvqlkesgpgsvapsqlsitctvsgfsltyvgvnnwvrppgkglwdlmiwgdgstdynsalksrslskdnksqvfl
kmdslqddtaryycardhygthyamdywgqgtsvtvssdq
25

59. FC2-2 VH L11S scFv

Nucleotide sequence:

gttgtaagcttgccgccatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt
30 cacagctcctatcctcctagctgtgtcagttggagagaagggttctatgagctgcaagtccagtcagagcctttatataatcacatca
aaagaactacttgccctggtagcagataaccagggcagctctcctaaactgctgattactgggcatccactagggaatctggggtc
cctgatcgcttcacaggcagtgatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg
tcagcaatattatcctatcctccacgttcggaggtggcaccaagctggaaataaaagggtggcgggtggcgggtgggtgggtc
gggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggctcggcggcgcctcacagagcctgtccatcacatgc
35 accgtctcaggggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatg
gggtgatggaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagtttcttaaaa
atggacagtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtc
aaggaacctcagtcaccgtctcctctgatcag

40 Amino acid sequence:

Mdsqaqvlmllllwwsgtcgdivmsqspsslavsvgekvsmckssqsllynhnqnknylawyqqipgqspklliywastres
gvpdrftgsgsgtdfltlisrvkaedlavyyccqyytypptfgggtkleikgggsgggsgggssqvqlkesgpgsvapsql
sitctvsgfsltyvgvnnwvrppgkglwdlmiwgdgstdynsalksrslskdnksqvflkmdslqddtaryycardhygt
hyamdywgqgtsvtvssdq
45

60. FC2-2 (SSS-S)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttgccgcatggattcacaggcccaggttctatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt
cacagcttccatcctccctagctgtgtcagttggagagaagggttctatgagctgcaagtcagtcagagcctttatataatcacatca
aaagaactacttggcctggtaccagcagataccaggggcagtcctctaaactgctgatttactgggcatccactagggaatctggggtc
5 cctgatcgcttcacaggcagtgatctgggacagatttactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg
tcagcaatattatccctatcctccacgttcggagggtggcaccaagctggaaataaaagggtggcgggtggctcgggcgggtgggtgggc
gggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggcctgggtggcggcctcacagagcctgtccatcacatgc
accgtctcagggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaaagggtctggactggctgggaatgatag
gggtgatggaagcacagactataattcagctctcaatccagactgagcatcagtaaggacaactccaagagccaagttttcttaaaa
10 atggacagctctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtc
aaggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtcctcagcacctgaact
cctgggtggaccgtcagcttctcttcccccaaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtg
gtggacgtgagccacgaagacctgaggtcaagttcaactgtgtacgtggacggcgtggaggtgcataatgccaagacaaagccgc
gggaggagcagtagacaacagcacgtaccgtgtgtgcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa
15 gtgcaaggcttccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt
gtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccaagcgaca
tcgccgtggagtgggagagcaatgggcagccgggagaaactacaagaccacgctcctcgtgctggactccgacggctccttctt
cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctatgctccgtgatgatgaggtctgcaca
accactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

20

Amino acid sequence:

mdsqavqlmlllwvsgtcgdivmsqspsslavsvgekvsmckssqsllynhnqnknylawyqqipqgspklliywastres
gvprdfitgsgsgtdflitirvkaedlavyyccqyyttypptfgggtkleikgggsgggsgggsgggssqvlkesgplvapsql
sitctvsgfltyvgvwnvrppgkglwdlwmigwdgstdynsalksrslskdnskqvflkmdslqtdtdaryycardhygt
25 hyamdywgqgtsvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmsirtpevtcvvdvshedpevkfnwy
vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltkn
qvsltclvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsisp
gk

30 61. FC2-2 VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttgccgcatggattcacaggcccaggttctatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt
cacagcttccatcctccctagctgtgtcagttggagagaagggttctatgagctgcaagtcagtcagagcctttatataatcacatca
aaagaactacttggcctggtaccagcagataccaggggcagtcctctaaactgctgatttactgggcatccactagggaatctggggtc
35 cctgatcgcttcacaggcagtgatctgggacagatttactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg
tcagcaatattatccctatcctccacgttcggagggtggcaccaagctggaaataaaagggtggcgggtggctcgggcgggtgggtgggc
gggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggctcgggtggcggcctcacagagcctgtccatcacatgc
accgtctcagggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaaagggtctggactggctgggaatgatag
gggtgatggaagcacagactataattcagctctcaatccagactgagcatcagtaaggacaactccaagagccaagttttcttaaaa
40 atggacagctctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtc
aaggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtcctcagcacctgaact
cctgggtggaccgtcagcttctcttcccccaaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtg
gtggacgtgagccacgaagacctgaggtcaagttcaactgtgtacgtggacggcgtggaggtgcataatgccaagacaaagccgc
gggaggagcagtagacaacagcacgtaccgtgtgtgcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa
45 gtgcaaggcttccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt
gtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccaagcgaca

tcgccgtggagtgaggagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgacggctccttctt
cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcaca
accactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

5 Amino acid sequence:

mdsqaqvlmlllwvsgtcgdivmsqspsslavsvgekvsmsckssqsllynhnqknylawyqqipgqspklliywastres
gvpdrftgsgsgtdfltisrvkaedlavyyccqyytypptfgggtkleikggggsgggsgggssqvqlkesgpgsvapsqsl
sitctvsgfsltygvnwvrqppgkgldwlgmiwgdgstdynsalksrlsiskdnsksqvlkmdslqddtaryycardhygt
hyamdywgqgtsvtvssdqepkssdkhtsppsapellggpsvflfppkpkdtlmisrtpcvvvdvshedpevkfnwy
10 vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltkn
qvsltclvkgyfypsdiavewesngqpennyktpvpldsdflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp
gk

62. UCHL-1 VH

15 Nucleotide sequence:

atgggcaggcttacttctcattcctgctactgattgttctgcatatgtcctctcccagattactctgaaagagcttggccctgggatcttg
cagccctcccagaccctcagctgacttgttcttctctgggtttcactgaccactatggtataggagtaggttgattcgtcagcctcc
aggggaagggtctggagtggtgacacacatttggtggaatgataataagtactataacacagccctgaggagccggctcacaatctc
caaggattcctccaacaaccaagtactcctcaagatcgccaatgtggacactgcagataccgccacatactactgtctctacggctac
20 acttactggggccaagggactctgtgactgtctctgca

Amino acid sequence:

mgrltssflilivpayvlsqitlkesgpgilqpsqtsltsfsgfsltyigvgwirppgkglewlthiwwndnkyyntalrsrli
skdssnnqvllkianvdtadtatyyclygytywggtlvtvsa
25

63. UCHL-1 VL

Nucleotide sequence:

atgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgacccaaactccactctccctgcc
tgtcagcttggagatcaggcctccatctcttcagatctagtcagagcctctttacagtaatggaaacacctattacattggtacctgc
30 agaagccaggccagcttccaaaactcctgatctacaaactttccaaccgatttctgggggtcccagacaggttcagtggcagtgatc
agggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctctcaaagtacacatgttccgtgga
cgttcggtggaggcaccaagctggaaatcaaa

Amino acid sequence:

35 mklpvrllvlfwipasisdvmtqtplslpvsldqasiscrssqsllysnngntylhwylqkpgqspklliyklsnrfsgvpdrf
sgsgsgtdftlkisrveadlgvyfcsqsthvpwtfgggtkleik

64. UCHL-1 scFv

40 Nucleotide sequence:

gttgtaagcttgccgccatgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgaccca
aactccactctccctgctgctcagcttggagatcaggcctccatctcttcagatctagtcagagcctctttacagtaatggaaacacc
tatttacattggtacctgcagaagccaggccagctcctcaaaactcctgatctacaaactttccaaccgatttctgggggtcccagacagg
ttcagtggcagtgatcaggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctctcaaa
45 gtacacatgttccgtggacgttcggtggaggaccaagctggaaatcaaatggcgggtggctgggaggtggatctggagg
aggtgggagctctcagattactctgaaagagctctggccctgggactctgcagccctcccagaccctcagctgacttgttcttctctgg

gtttcactgaccacttatggtataggagtaggttgattcgtcagcctccaggaagggtctggagtggctgacacacatttggtgga
atgataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcctcaagatcgc
caatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctgtcactgtctctgctg
atca

5

Amino acid sequence:

mklpvrllvImfwipasisdvmtqtplslpvsldqasiscrssqsllysnngtylhwylqkpgqspklliyklsnrfsgvpdrf
sgsgsgtdfIlkisrveaedlgvyfcsqsthvpwtfgggkldggsgggsgggssqitlkesgpgilqpsqtlsltsfsgf
sltygigvgwirppgkglewlthiwwndnkyyntalrsrltiskdssnnqvllkianvdtadtatyyclygytywgqglvtvs
ad

10

65. UCHL-1 VH I11SL12S

Nucleotide sequence:

15 gggagctctcagattactctgaaagagtctggccctgggatcttgagccctcccagaccctcagctgactgttctttcttggtttt
cactgaccacttatggtataggagtaggttgattcgtcagcctccaggaagggtctggagtggctgacacacatttggtggaatga
taataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcctcaagatcgccaat
gtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctgtcactgtctctgctgatca

20

Amino acid sequence:

(gss)qitlkesgpgssqpsqtlsltsfsgfsltygigvgwirppgkglewlthiwwndnkyyntalrsrltiskdssnnqvllki
anvdtadtatyyclygytywgqglvtvsad

25

66. UCHL-1 scFv VH L11S

Nucleotide sequence:

gttgtaagcttgccgcatgaagttgcctgtaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgaccca
aactccactctccctgcctgtcagcttggagatcaggcctccatcttgcagatctagtcagagccttcttacagtaatggaacacc
tatttacattggtacctgcagaagccaggccagctctccaaactcctgatctacaaacttccaaccgattttctggggctccagacagg
30 ttcagtggcagtgatcagggacagatttcacactcaagatcagcagagtgaggctgaggatctgggagttatttctgctctcaaa
gtacacatgttcctgtgacgttcggtggagggaccaagctgaaatcaaatggcgggtggctcgggcgggtggtgatctggagg
aggtgggagctctcagattactctgaaagagtctggccctgggagctcccagccctcccagaccctcagctgactgttctttctctg
ggtttctactgaccacttatggtataggagtaggttgattcgtcagcctccaggaagggtctggagtggctgacacacatttggtgg
aatgataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcctcaagatcg
35 ccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctgtcactgtctctgct
gatca

Amino acid sequence:

40 mklpvrllvImfwipasisdvmtqtplslpvsldqasiscrssqsllysnngtylhwylqkpgqspklliyklsnrfsgvpdrf
sgsgsgtdfIlkisrveaedlgvyfcsqsthvpwtfgggkldggsgggsgggssqitlkesgpgssqpsqtlsltsfsgf
sltygigvgwirppgkglewlthiwwndnkyyntalrsrltiskdssnnqvllkianvdtadtatyyclygytywgqglvtvs
ad

45

67. UCHL-1 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttgccgccatgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgaccca
aactccactctccctgcctgtcagcttggagatcaggccctccatctcttcagatctagtcagagccttcttacagtaatggaacacc
tatttacattggtacctgcagaagccaggccagctcctcaaaactcctgatctacaaactttccaaaccgattttctggggtcccagacagg
5 ttcagtggcagtggtacaggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgcttcaaa
gtacacatgttccgtggacgttcggtggaggcaccaagctggaaatcaaagatggcggtggctcgggcgggtggtggatctggagg
aggtgggagctctcagattactctgaaagagtctggccctgggatcttcagccctcccagaccctcagctcgtactgttcttctctgg
gttttactgaccacttatggtataggagtaggttgattcgtcagcctccagggaagggtctggagtggctgacacacatttggtgga
atgataataagtactataacacagccctgaggagccggtcacaatctccaaggattcctccaacaaccaagtactcctcaagatcgc
caatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggctactgtctctgctg
10 atcaggagcccaaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggtggaccgtcagcttctctctc
ccccaaaaaccaaggacaccctcatgatctcccgaccctgagggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttaactggtacgtggacggcgtggagggtgcataatgccaaagacaaagccgcgggaggagcagtacaacagcacgt
accgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaagccctc
ccagcccccatcgagaaaaccatctccaaagccaaaggcagccccgagaaccacaggtgtacaccctgccccatcccgggat
15 gagctgaccaagaaccaggtcagcctgacctgcctggtaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg
ggcagccgggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttctctctacagcaagctcaccgtggac
aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc
cctgtctccgggtaaatgatctaga

20 Amino acid sequence:
mklpvrllvmlfwipasisdvmtqtplslpvsldqasiscrssqsllysnngtylhwyqlkpgqspklliylksnrfsgvpdrf
sgsgsgtdflkisirveadlgyvfcsqsthvpwtfgggtkleikdggsgggsgggssqitkesgpilqpsqtslscsfsgf
slttygigvgwirppgkglewlthiwwndnkyyntalrslrskdssnnqvllkianvdtadtatyyclygytywqggtlvts
adqepkssdkthtspssapellggpsvflfppkpkdtlmisrtpetcvvvdvshedpevkfnwyvdgvevhnaktkpreeq
25 ynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvslclvkgyfypsdiave
wesngqpennyktpvpvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslspsgk

68. UCHL-1 scFv VHL11S (SSS-S)H WCH2 WCH3

30 Nucleotide sequence:
gttgtaagcttgccgccatgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgaccca
aactccactctccctgcctgtcagcttggagatcaggccctccatctcttcagatctagtcagagccttcttacagtaatggaacacc
tatttacattggtacctgcagaagccaggccagctcctcaaaactcctgatctacaaactttccaaaccgattttctggggtcccagacagg
ttcagtggcagtggtacaggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgcttcaaa
35 gtacacatgttccgtggacgttcggtggaggcaccaagctggaaatcaaagatggcggtggctcgggcgggtggtggatctggagg
aggtgggagctctcagattactctgaaagagtctggccctgggagctcccagccctcccagaccctcagctcgtactgttcttctctg
ggttttactgaccacttatggtataggagtaggttgattcgtcagcctccagggaagggtctggagtggctgacacacatttggtgg
aatgataataagtactataacacagccctgaggagccggtcacaatctccaaggattcctccaacaaccaagtactcctcaagatcg
ccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggctactgtctctgct
40 gatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggtggaccgtcagcttctctctt
ccccaaaaaccaaggacaccctcatgatctcccgaccctgagggtcacatgcgtggtggtggacgtgagccacgaagaccct
gaggtcaagttcaactggtacgtggacggcgtggagggtgcataatgccaaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaagccct
cccagccccatcgagaaaaccatctccaaagccaaaggcagccccgagaaccacaggtgtacaccctgccccatcccggga
45 tgagctgaccaagaaccaggtcagcctgacctgcctggtaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg
ggcagccgggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttctctctacagcaagctcaccgtggac

aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc
cctgtctccggtaaatagatctagaa

Amino acid sequence:

5 mklpvrlvlmfwipasisdvmtqtplslpvsldqasiscrssqsllysnngtylhwylqkpgqspklliyklsnrfsgvprf
sgsgsgtdftlkisrveadlgvyfcsqsthvpwtfgggkgleikdggsgsgsgsgsgsgssqitlkesgpgssqpsqtlstcsfsgf
slttygigvgwirppgkglewlthiwwndnkyyntalrsrltiskdssnnqvllkianvdtadtatyyclygytywgqgtlvtvs
adqepkssdkthtspssapellggspsvflfppkpkdtlmisrtpvctvvvdvshedpevkfnwyvdgvevhnaktkpreeq
10 ynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltclvkgfypsdiave
wesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispk

69. 5B9 VH

Nucleotide sequence:

15 atggctgtcttggggctgctcttctgcctggtagacatttccaagctgtgtcctatcccagggtgcagctgaagcagtcaggacctggcct
agtgcagtcctcacagagcctgtccatcacctgcacagctctctggtttctcattaactacatgctgtacactgggttcgccagctcca
ggaaagggctggagtggtggagtgatggagtggtggaatcacagactataatgcagcttccatccagactgagcatcacc
aaggacgattccaagagccaagtttctttaaaatgaacagctgcacacctaataacacagccatttattactgtgccagaaatgggggt
gataactacccttattactatgctatggactactggggtcaaggaacctcagtcaccgtctcctca

20

Amino acid sequence:

mavlgllfclvtfpscvlsqvqlkqsgpglvqssqslsitctvsgfslttyavhwvrqspgkglewlgviwsggitdynaafisrlsi
tkddsksqvfikmnsllqpnndtaiyycaarnggdnyppyyamdywgqgtsvtvss

25 70. 5B9 VL

Nucleotide sequence:

atgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgcaggctgcattctcca
atccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagctcctacatagtaatggcatcacttattgtattgtatc
tgcagaagccaggccagctcctcagctcctgatttatcagatgtccaacctgcctcaggagtcaggagagcaggttcagtagcagtggt
30 gtcaggaaactgattcacactgagaatcagcagagtgagggtgaggatgtgggtgttattactgtgctcaaaatctagaactccgct
cacgttcggtgctgggaccaagctggagctgaaacgg

Amino acid sequence:

35 mrfsaqllgllvlwipgstadivmtqaafsnpvtlgtssasiscrskslhsngitylywylqkpgqspqlliyqmsnlasgvprf
sssgsgtdftlrisrveadvgyycaqnlelpltfaggtkglekr

71. 5B9scFv

Nucleotide sequence:

40 Aagcttgcggccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcacttctggaacatcagcttccatctcctgcaggtctagtaagagctcctacatagtaatggcatcact
tattgtattggtatctgcagaagccaggccagctcctcagctcctgatttatcagatgtccaacctgcctcaggagtcaggagagcaggt
tcagtagcagtggtcaggaactgattcacactgagaatcagcagagtgagggtgaggatgtgggtgttattactgtgctcaaaat
ctagaactccgctcacgttcggtgctgggaccaagctggagctgaaacgggggtggcgggtggctcggcggtggtgggtcgggtg
gcggcgggagctctcagtgacagtgagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtc
45 tctggttctcattaactacatgctgtacactgggttcgccagctcctcaggaaagggtctggagtggtgggagtgatggagtggt
ggaatcacagactataatgcagcttccatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaaatgaacagt

ctgcaacctaatagacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggggtcaa
ggaacctcagtcaccgtctcctcag

Amino acid sequence:

5 mrfsaqlglglvlwipgstadivmtqaafsnpvtlgtasiscrskslhsngitylywylqkpgqspqlliyqmsnlasgvpdrf
sssgsgtdflrisrveadvgyycaqnlelpltfagtklelkrgggsgggsgggssqvqlkqsgpglvqssqslsitctvs
gflsttyavhwvrqspgkglewlgviwsggitdynaafisrlsitkddsksqvffkmnslqpndtaiyyccarnggdnyppyya
mdywggqtsvtvss

10 72. 5B9 VH L11S

Nucleotide sequence:

gggagctctcaggtgcagctgaagcagtcaggacctggctcagtcagtcctcacagagcctgtccatcacctgcacagtccttggt
ttctcattaactacatgctgtacactgggttcgccagtcctcaggaaagggcttgagtggtgggagtgatatggagtggtggaat
cacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttcttaaaatgaacagtcgca
15 acctaatagacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggggtcaaggaac
ctcagtcaccgtctcctcag

Amino acid sequence:

20 (gss)qvqlkqsgpgsvqssqslsitctvs gflsttyavhwvrqspgkglewlgviwsggitdynaafisrlsitkddsksqvffk
mnslqpndtaiyyccarnggdnyppyyamdywggqtsvtvss

73. 5B9 VH L11S scFv

Nucleotide sequence:

25 aagcttgccgccatgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgcag
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta
tttgattggtatctgcagaagccaggccagtcctcagtcctgatttatcagatgtccaacctgcctcaggagtcacagaggttc
agtagcagtgggtcaggaactgattcacactgagaatcagcagagtgaggctgaggatgtgggtgtttattactgtgctcaaaatct
agaacttccgctcacgttcgggtgctgggaccaagctggagctgaaacgggggtggcgggtggctcgggcgggtgggtgggtcgggtgg
30 cggcgggagctctcaggtgcagctgaagcagtcaggacctggctcagtcagtcctcacagagcctgtccatcacctgcacagtc
ctggtttctcattaactacatgctgtacactgggttcgccagtcctcaggaaagggcttgagtggtgggagtgatatggagtggt
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttcttaaaatgaacag
ctgcaacctaatagacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggggtcaa
ggaacctcagtcaccgtctcctcag

35

Amino acid sequence:

mrfsaqlglglvlwipgstadivmtqaafsnpvtlgtasiscrskslhsngitylywylqkpgqspqlliyqmsnlasgvpdrf
sssgsgtdflrisrveadvgyycaqnlelpltfagtklelkrgggsgggsgggssqvqlkqsgpgsvqssqslsitctvs
gflsttyavhwvrqspgkglewlgviwsggitdynaafisrlsitkddsksqvffkmnslqpndtaiyyccarnggdnyppyya
40 mdywggqtsvtvss

73. 5B9 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence:

45 aagcttgccgccatgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgcag
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta

5 tttgtattggtatctgcagaagccaggccagctctcctcagctcctgatttatcagatgtccaaccttgccctcaggagtcacagacaggttc
 agtagcagtggtgcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgtttactgtgctcaaaatct
 agaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcgggtggtcgggcgggtggtgggtcgggtgg
 cggcgggagctctcaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagctc
 10 ctggtttctcattaactacctatgctgtacactgggttcgccagtaaggggtctggagtggctgggagtgatatggagtgggt
 ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt
 ctgcaacctaatgacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa
 ggaacctcagtcaccgtctcctctgatcaggagccccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcct
 ggggtggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgggt
 15 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
 ggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctaccgtctgcaccaggactgggtgaatggcaaggagtacaag
 tgcaaggttccaacaaagccctcccagccccatcgagaaaaccatctcaaagccaaagggcagccccgagaaccacaggtgt
 acacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatccaagcgacatc
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttctcc
 20 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
 cactacacgcagaagagcctctccctgtctccgggtaaatgatctagag

Amino acid sequence:

20 mrfsaqlllvlwipgstadivmtqaafsnpvtlgtssisrskslhsngitylywylqkpgqspqlliyqmsnlasgvprf
 ssssgtdflrisrveaedvgvycaqnlepltfagtklelkrsgsgsgsgsgssqvqlkqsgpglvqssqslsitctvs
 gfslltyavhwvrqspgkglewlgviwsggtdynaafisrlsitkddsksqvffkmnslqpndtaiyyarnngdnyppyya
 mdywgqgtsvtvssdqepkssdkthtspssapellggsvglfpkpkdltlmisrtpvtecvvdvshedpevkfnwyvdg
 vevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsit
 25 clvkgyfypsdiavewesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

74. 5B9 scFv VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

30 aagcttgcgccatgaggttctctgctcagcttctggggctgcttctgctctggatccctggatccactgcagatattgtgatgacgcag
 gctgcattctccatccagtcactcttgaacatcagcttccatctcctgcagggtctagtaagagtctcctacatagtaatggcatcactta
 tttgtattggtatctgcagaagccaggccagctctcctcagctcctgatttatcagatgtccaaccttgccctcaggagtcacagacaggttc
 agtagcagtggtgcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgtttactgtgctcaaaatct
 agaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcgggtggtcgggcgggtggtgggtcgggtgg
 cggcgggagctctcaggtgcagctgaagcagtcaggacctggctcagtcagtcctcacagagcctgtccatcacctgcacagctc
 ctggtttctcattaactacctatgctgtacactgggttcgccagtaaggggtctggagtggctgggagtgatatggagtgggt
 35 ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt
 ctgcaacctaatgacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa
 ggaacctcagtcaccgtctcctctgatcaggagccccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcct
 ggggtggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgggt
 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
 40 ggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctaccgtctgcaccaggactgggtgaatggcaaggagtacaag
 tgcaaggttccaacaaagccctcccagccccatcgagaaaaccatctcaaagccaaagggcagccccgagaaccacaggtgt
 acacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatccaagcgacatc
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttctcc
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
 45 cactacacgcagaagagcctctccctgtctccgggtaaatgatctagag

Amino acid sequence:

mrfsaqlglglvlpwpgstadvmtqaafsnvptlgtasiscrskslhsngitylywylqkpgqspqliyqmsnlasgvpdrf
sssgsgtdflrisrveadvgyycaqnlelpltfagtklelkrggsgggsgggsgssqvqlkqsgpgsvqssqslsitctvs
gfslttyavhwvrqspgkglewlgviwsggtdynaafisrlsitkddsksqvffkmnslqpndtaiyyarnngdnyppyya
5 mdywggqgtsvtvssdqepkssdkthtsppssapellggpsvflfpkpkdltlmisrtpevtcvvvdvshedpevkfnwyvdg
vevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsit
clvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispkg

75. 2H7 scFv (SSS-S)H P238SCH2 WCH3

10 Nucleotide sequence:

aagcttgccgcatggaatttcaagtcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
15 ccaccacgtcgggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggtgagctggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatctccagcagcactacatgcagctcagcagcctgac
atctgaagactctcgggtctattctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaccac
20 ggtcaccgtctctctgatcaggagcccaaatctctgacaaaactcacacatcccaccgtctcagcacctgaactcctgggggga
tcgtcagctcttcttccccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatcggtggtggagctga
gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggagagc
agtacaacagcagctaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc
tccaacaaagccctcccagccccatcgagaaaacaatccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
25 ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcctgtagcatgaggctctgcacaaccactacacg
cagaagagcctctccctgtctccgggtaaatgatctaga

30 Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
sgsgtsysltisrveadaatyycqqsfnpptfagtklelkdggsgggsgggsgssqaylqqsgaelvrpgasvkmckas
gytftsynmhwwkqtrqglewigaiypngdtsynqkfkgkatltvdksstaymqlssltedsavvfcarvvyysnsywy
fdvwtgtttvtvssdqepkssdkthtsppssapellggssvflfpkpkdltlmisrtpevtcvvvdvshedpevkfnwyvdgve
35 vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitcl
vkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispkg

76. 2H7 scFv VH L11S (SSS-S)H P238SCH2 WCH3

Nucleotide sequence:

40 aagcttgccgcatggaatttcaagtcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagtacatgcactgg
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga
gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgtcgggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc
45 tctcaggcttatctacagcagctctggggtgagtcggtagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact

5
10

tcctacaatcagaagttcaaggggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggctactcgcgatgtctggggcacaggggaccac
ggtcaccgtctcttctgatcaggagcccaatcttctgacaaaactcacacatcccaccgtcctcagcacctgaactcctgggggga
tcgtcagtttctcttcccccaaaaccaaggacaccctcatgatctccggaccctgaggtcacatgcgtgggtggtagtga
gccacgaagaccctgaggtcaagttcaactggtagctggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc
tccaacaaagccctcccagccccatcgagaaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctg
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtggactccgacggctccttcttctctacagca
agctcaccgtggacaagagcaggtggcagcagggggaacgtcttctcatgtcctcgatgcatgaggctctgcacaaccactacagc
cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvmtmtcrasssvsymhwyqqkpgsspkpwiayapsnlasgvparfsg
sgsgtsysltisrveadaatyycqqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsymbhvwkqtprrglewigaiypngdtsynqkfkkgatltdkssstaymqlssltedsavyfcarvvyyysnsywy
fdvwgtgtvtvssdqepkssdkthtspssapellgsssvflfppkpkdtlmiistrpevtcvvvdvshedpevkfnwyvdgve
vhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitcl
vkgfypsdiavewesngqpennyktthppvldsdgsffllyskltvdksrwqqgnvfscsvmhhealhnhytqkslslspgk

77. 2H7 scFv IgA WH WCH2 WCH3 + J Chain

Nucleotide sequence:

2H7 scFv IgA WH WCH2 WCH3

aagcttgcgcgcacatggattttcaagtgacagattttcagcttcttgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgcagggccagctcaagtgtaatgtacatgcactgg
taccagcagaagccaggatcctccccaaaccttgatttatgccccatccaacctggcttctggagtcctgtctgcttcagtgga
gtgggtctgggaccttactctctcacaaatcagcagagtggagggtgaagatgctgccacttattactgccagcagtggaagttaaac
ccaccacgcttcgggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtgggtggatctggaggaggtgggagc
tctcaggttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtggaagatgtctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcacaggggaccac
ggtcaccgtctcttctgatcagccagttccctcaactccacctacccccatctccctcaactccacctacccccatctccctcatgctgccac
ccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag
agatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctgacctctgtggctg
ctacagcgtgtccagtgctctgccgggctgtgccgagccatggaacctgggaagaccttcacttgcactgctgcctaccccgagt
caagaccccgctaaccgccacctctcaaaatccggaacacattccggcccaggtccacctgctgccgccgccgtcggaggag
ctggccctgaacgagctgggtgacgtgacgtgcctggcacgtggcttcagccccaggatgtgctggttcgtggctgcaggggtc
acaggagctgccccgcgagaagtacctgacttgggcatccggcgaggagccagggcaccaccaccttcgctgtgaccag
catactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttc
acacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgttgcattggcgagggtggacggcacctgtctac
tgataatctaga

+J Chain

agatctcaagaagatgaaaggattgtcttgttgacaacaaatgtaagtgtgccggattacttccaggatcatccgttctccgaagatc
ctaatagaggacattgtggagagaaacatccgaattattgttctctgaacaacagggagaatatctctgatccacctcaccattgaga

accagatttggtaccatttgctgacctcagctgtaaaaaatgtgacctacagaagtggagctggataatcagatagttactgctaccc
agagcaatatctgtgatgaagacagtgtacagagacctgtacacttatgacagaaacaagtgtacacagctgtgggtccactcgt
atatgggtggtgagaccaaagtgtggaacagccctaaccccagatgcctgctatcctgactaatctaga

5 Amino acid sequence:

2H7 scFv IgA WH WCH2 WCH3

mdfqvqifsfllisasviiargqivlsqspailsaspgekvmttcrasssvsymhwyyqkpggsspkpwiypasnlasgvparfsg
sgsgtsysltsirveadaatyycqqwsfnpptfgagtklelkdggsgsgggsgggssqaylqqsgaelvrpgasvkmsckas
gytftsynmhvkwqtpqrqglewigaiypngdtsynqfkfkgkatltvdkssstaymqlssltedsavyfcarvvyyysnsywy
fdvwgtgtvtvssdqpvpstpptpspstpspcchprlslhrrpaledlllseailtctltglrdasgytftwtpssgksavqgp
pdrdlcgcysvssvlpgcaepwnhgtkftctaaypesktptlatlsksgntfrpevhllpppseelalnelvtltclargfspkdvivr
wlqsgqlprekyltwasrqepsqgtttfavtsilrvaedwkkgdtfscmvghealplafqtktidrlagkpthvnvsvvmaev
dgtcy

15 +J Chain

rsqederivldnkckcaritsriirssedpnediverniriivplnnrenisdptsplrtrfvyhlsdlsckkcdptevelndnqivtatq
snicdedsatetcytydmkcytavvplvyggetkmvetaltpdacypd

20

78. 2H7 scFv VH L11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

25 aagcttgccgccaatggattttcaagtgcagattttcagcttccgtctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctcagcaaatcctgtctgcattctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagttacatgcactgg
taccagcagaagccaggaatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtggca
30 gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtttaac
ccaccacgttcggtgctgggaccaagctggagctgaagatggcgggtggctcgggcgggtggtgatctggaggaggtgggagc
tctcaggttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgctcctgcaaggcttctggctacacat
ttaccaggtacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
35 tctacaatcagaagttaaggggcaaggccacactgactgtagacaaaatctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctctctgatcaggagcccaatctctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga
ccgtcagcttctcttccccccaaaacccaaggacacctcatgatctccggaccctgagggtcacatgcgtggtggtggacgtga
gccacgaagaccctgagggtcaagttcaactggtagctggacggcgtggagggtgcataatgccaagacaaagccgcgggaggagc
40 agtacaacagcagctaccgtgtggtcagcgtctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggct
tccaacaaagccctcccagccccatcgagaaaaaatctccaaagccaaagggcgacccccgagaaccacaggtgtacaccctg
ccccatcccgggatgagctgaccaagaaccagggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaaactacaagaccagcctcccgtgtggtgactccgacggctccttcttctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgtcctgtagcatgaggctctgcacaaccactacacg
45 cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

45 mdfqvqifsflilasviiargqivlsqspailsaspgkvmtcrassvsymhwyyqkpgsspkpwiypasnlasgvparfsg
sgsgtsysltisrveadaatyycqqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsymnhwvkqtrpqglewigaiypngdtsynqkfkgaatltdkssstaymqlslltsedsavyfcarvvyysnsywy
fdvwgtgtvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv

evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltc
lvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslsispkg

5 **79. 2H7 scFv VH L11S (CSS-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggattttcaagtcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgaagttacatgcactgg
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca
10 gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtgagatcgaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgctcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcgctggaatggattggagctattatccaggaaatggtgatact
tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
15 atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacaggggaccac
ggtcaccgtctcttctgatcaggagcccaaatcttggacaaaactcacacatcccaccgtcctcagcacctgaactcctgggggga
ccgtcagcttctcttcccccaaaaccaaggacacctcatgatctccggaccttgaggtcacatgcgtggtggtggacgtga
gccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgaggagc
agtacaacagcagctaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc
20 tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctg
ccccatcccggtgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctggtgactccgacggctccttctctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacg
cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwi yapsnlasgvparfsg
sgsgtsysltisrveaadaatyycqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavvyfcarvvyysnsywy
30 fdvwgtgtvtvssdqepkscdkthtspssapellggpsvflfppkpkdtlmisrtpetcvvvdvshedpevkfnwyvdgv
evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltc
lvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslsispkg

78. G8-1 scFv VHL11S (SCS-S)H WCH2 WCH3

Nucleotide sequence:

ggtgtaagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatg
actcagctcagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgttacagttatttgctt
ggatcagcagaaacagggaaaatctctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgcatcaaggttcagtg
cagtggtatcaggcacacagtttcttgaagatcagcagcctgcagcctgaagattctggaagttattctgtcaacatcattccgataat
40 ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggtcggcggtggtgggtcgggtggcggcggtatc
gtcagcgggtccagctgcagcagcttgacctgagtcgaaaagcctggcgcttcagtgaaagttctgcaaggtctctggtactca
ttcactggctacaatatgaactgggtgaagcagaataatggaagagccttgagtggattggaatatgtatcttattatggtggtact
acctacaaccggaagtcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgac
atctgaggactctgcagcttattactgtgcaagatcggtcggccctatggactactgggggtcaaggaaacctcagtcaccgtctcttctg
45 atcaggagcccaaatcttctgacaaaactcacacatgccaccgtcctcagcacctgaactcctgggtggaccgtcagcttctcttc
ccccaaaaccaaggacacctcatgatctccggacctgaggtcacatgcgtggtggtggacgtgagccacgaagacctg

aggtcaagttcaactgtgtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgt
 accgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctccaacaagccctc
 ccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccggtat
 gagctgaccaagaaccaggtcagcctgacctgcctggctcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg
 5 ggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggac
 aagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc
 cctgtctccgggtaaatgatctagag

Amino acid sequence:

10 mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsfgsgs
 gtqfslkisslqpedsgsyfcqhhsdnptwfgggteleikggggsgggsgggssavqlqsgpesekpgasvkiskcasgys
 ftgynmnwvkqnnkslewnidpyyggtynrkfkgtatltvdksstaymqlksltsedsavyycarsvgpmdywgqg
 tsvtvssdqepkssdkthtccppsapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnakt
 kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrldeltnqvsltlvkgyfyp
 15 sdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhnhytqkslsispkg

79.. G28-1 scFv VHL11S (CCS-P)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatg
 20 actcagctcagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgttacagttatttgctt
 ggtatcagcagaacagggaatatctcctcagctcctgtctcttttgcacaaaccttagcagaaggtgtgccatcaaggttcagtg
 cagtggtatcaggcacacagttttcttgaagatcagcagcctgcagcctgaagattctggaagttattctgtaacatcattccgataat
 ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggatc
 gtcagcgggtccagctgcagcagcttgacctgagtcggaaaagcctggcgcttcagtgaagatttctgcaaggttctgtgtactca
 25 ttcactggctacaatatgaactgggtgaagcagaataatgaaagagccttgatgtgattgaaatattgatccttattatggtgtact
 acctacaaccggaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagcttgac
 atctgaggactctgcagcttattactgtgaagatcggtcggccctatggactactggggtaaggaaacctcagtcaccgtctctctg
 atcaggagcccaaatctgtgacaaaactcacatgtccaccgtccccagcactgaactcctgggtggaccgtcagcttctctctc
 ccccaaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtgggtgggtggacgtgagccacgaagacctg
 30 aggtcaagttcaactgtgtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgt
 accgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaagccctc
 ccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccggtat
 gagctgaccaagaaccaggtcagcctgacctgcctggctcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg
 ggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggac
 35 aagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc
 cctgtctccgggtaaatgatctaga

Amino acid sequence:

40 mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsfgsgs
 gtqfslkisslqpedsgsyfcqhhsdnptwfgggteleikggggsgggsgggssavqlqsgpesekpgasvkiskcasgys
 ftgynmnwvkqnnkslewnidpyyggtynrkfkgtatltvdksstaymqlksltsedsavyycarsvgpmdywgqg
 tsvtvssdqepkscdkthtccppsapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnakt
 kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrldeltnqvsltlvkgyfyp
 sdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhnhytqkslsispkg

45

80. G28-1 scFv VH L11S (SCC-P)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatg
actcagctcagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttgctt
ggtatcagcagaaacagggaaaatctcctcagctcctggtctcttttgcataaaccttagcagaaggtgtgccatcaagggtcagtg
5 cagtgatcagggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtaacatcattccgataat
ccgtggacgttcggtggagggaccgaactggagatcaaagggtggcgggtggctcgggcgggtgggtggcggcggtgac
gtcagcgggtccagctgcagcagctgtgacctgagtcggaaaagcctggcgcttcagtgaaagatttctgcaaggcttctggttactca
ttcactggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaaatattgatccttattatggtggtact
acctacaaccggaaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgac
10 atctgaggactctgcagctctattactgtgcaagatcggtcggccctatggactactggggtaagggaacctcagtcaccgtctcttctg
atcaggagcccaaatcttctgacaaaactcacatgccaccgtgccagcacctgaactcctgggtggaccgtcagcttctctt
cccccaaaacccaaggacaccctcatgatctcccgacccttgaggtcacatgcgtgggtgggtgacgtgagccacgaagaccct
gaggtcaagttcaactggtacgtggacggcggtgaggtgcataatgcaagacaaagccgaggaggagcagtagacaacagcacg
taccgtgtggtcagcgtcctcaccgtctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtctccaacaaagccct
15 cccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccggga
tgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg
ggcagccgggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttcttctctacagcaagctcaccgtggac
aagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgatgaggctctgcacaaccactacacgcagaagagccctc
cctgtctccgggtaaatgatctaga
20

Amino acid sequence:

mvstaqlflglwltggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsghsgs
gtqfslkisslqpedsgsyfcqhhsdnptwtfgggtelkkgggsgggsgggssavqlqsgpeseekpgasvkiskcasgys
ftgynmnwvkqnnkslewnidpyyggtynrkfkakatltvdksstaymqlksltsedsavyycarsvgpmdywgqg
25 tsvtvssdqepkssdkthtppcpapellggpsvflfppkpkdtlmisrtpetvvdvshedpevkfnwyvdgvevhnakt
kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrldeltnqvslclvkgyfyp
sdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

30 81. G28-1 scFv VH L11S mIgE CH2 CH3 CH4

Nucleotide sequence:

aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca
gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggttggtat
cagcagaaacagggaaaatctcctcagctcctggtctcttttgcataaaccttagcagaaggtgtgccatcaagggtcagtggcagtg
35 gatcagggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtaacatcattccgataatccgtg
gacgttcggtggagggaccgaactggagatcaaagggtggcgggtggctcgggcgggtgggtggcggcggtgacgtcag
cggtccagctgcagcagcttgacctgagtcggaaaagcctggcgcttcagtgaaagatttctgcaaggcttctggttactcattcact
ggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaaatattgatccttattatggtggtactacctac
aaccggaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctga
40 ggactctgcagctctattactgtgcaagatcggtcggccctatggactactggggtaagggaacctcagtcaccgtctcttctgatcacg
ttcgacctgtcaacatcactgagccaccttgagctactccattcatcctgcgaccccaatgcattccactccaccatccagctgtact
gcttcatttatggccacatcctaaatgatgtctctgcagctggctaattggacgatcgggagataactgatacattgcacaaactgttct
aatcaaggagggaaggcaactagcctctacctgcagtaaaccaacatcactgagcagcaatggatgtctgaaagcaccttcacctg
caaggtcacctccaaggcgtagactatttggccacactcggagatgccagatcatgaccacgggggtgtgattacctacctgat
45 cccaccagccccctggacctgtatcaaacgggtgtcccaagcttacctgtctggtgggtggacctggaaagcgagaagaatgtcaa
tgtgacgtggaaccaagagaagaagacattcagctcagcatccagtggtactaagcaccacaataacgccacaactagtatcac

83. G28-1 scFv Vh L11S hIgE CH2 CH3 CH4

Nucleotide sequence:

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca
5 gctccagcctccctatctgcatctgtgggagagactgtcacatcacatgtcgaacaagtgaatatgtttacagttatttggcttggat
cagcagaaacagggaatatctctcagctcctggctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg
gatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagttatttctgcaacatcattccgataatccgtg
gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcggcggttggtgggtggcgggcggatcgtcag
cggctcagctgcagcagcttgacctgagtcggaaaagcctggcgcttcagtgaagattcctgcaaggcttctggttactcattcact
10 ggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtggaattggaaatattgatccttattatggtggtactacctac
aaccgggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctgacatctga
ggactctgcagctattactgtgcaagatcggtcggccctatggactactgggtgcaaggaacctcagtcaccgtctcttctgatcacg
tctgtccagggtaccccccaccgtgaagatcttacagtcgtcctgcgacggcgggcggcacttcccccgacctccag
ctcctgtgctcgtctctgggtacacccaggactatcaacatcacctggctggaggacggcgaggtcatggacgtggactgtcc
15 accgcctctaccacgcaggagggtgagctggcctccacacaaaagcgagctcaccctcagccagaagcactggctgtcagaccgc
acctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattccaacccgagaggggtgag
cgctacctaagccggccagcccgttcgacctgttcacccgaagtcgcccacgatcacctgtctggtggtggacctggcaccag
caaggggaccgtgaacctgacctgggtcccggccagtggaagcctgtgaaccactccaccagaagggaggagaagcagcgca
atggcacgttaaccgtcacgtccacctgcccgggtgggacccgagactggatcgagggggagacctaccagtgcagggtgacct
20 acccccacctgccagggccctcatgctggtccacgaccaagaccagcgcccgcgtgctgccccggaagctatgcgttgcgac
gcccggagtggcggggagccgggacaagcgaccctgcctgctgatccagaacttcagctgaggacatctcggtgcagtg
gctgcacaacgaggtgcagctcccgacggcgccgacagcacgacgcagccccgaagaccaagggtccggcttcttctgttt
cagccgctggaggtgaccagggccgaatgggagcagaagatgagttcatctgccgtgcagtcctatgaggcagcgagccctc
acagaccgtccagcgagcgggtgtctgtaaatcccggtaaatgataatctaga

Amino acid sequence:

mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitcrtsenvysylawyqqkqkspqllvsfaktlaevpsrfsrgsgs
gtqfslkisslqpedsyfcqhhsdnptwfgggteleikgggsgggsgggssavqlqqsgpesekpgasvkiskcasgys
ftgynmnwvkqnngkslewignidpyyggtytnrkfkkgkatltvdksstaymqlsltsedsavyycarsvgpmdywgqg
30 tsvtvssdhvcsrdftpptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmdivlstatteqelastqseltsqk
hwlsdrtytcvtyqghtfedstkkcadsnprgvsaylsrpsfdlfrksptitclvdlapskgtvnlwsrasgkpvnhstrkee
kqrngltltvtlplvgtrdwiegetyqcrvthphlpralmrstktsgpraapevyafatpewpgsrdrklaciqnfmpedisvq
wlhnevqlpdarhsttqprktksgffvfrlevtraeweqkdeficravheaaspsqtvravsvnpgk

84. G28-1 scFv VH L11S hIgA WH WCH2 T4CH3

Nucleotide sequence:

atggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactcagctccagcctcc
ctatctgcatctgtgggagagactgtcacatcacatgtcgaacaagtgaatatgtttacagttatttggcttggatcagcagaaacag
40 ggaaaatctctcagctcctggctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggcagtggtatcaggcacac
agttttctctgaagatcagcagcctgcagcctgaagattctggaagttatttctgcaacatcattccgataatccgtggacgttcggtgg
aggcaccgaactggagatcaaaggtggcgggtggctcggcggttggtgggtggcgggcggatcgtcagcgggtccagctgc
agcagctctggacctgagtcggaaaagcctggcgcttcagtgaagattcctgcaaggcttctggttactcattcactggctacaatatg
aactgggtgaagcagaataatggaaagagccttgagtggaattggaaatattgatccttattatggtggtactacctacaaccggaagtt
45 caagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagcttgacatctgaggactctgcagt
ctattactgtgcaagatcggtcggccctatggactactgggtgcaaggaacctcagtcaccgtctcttctgatcagccagttccctcaa

ctccacctaccccatctccctcaactccacctaccccatctccctcatgctgccaccccgactgtcactgcaccgaccggccctcga
 ggacctgctcttaggtcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgcc
 ctaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgtacagcgtgtccagtgtcctgccgggctgtgc
 cgagccatggaacctgggaagaccttcaactgtcactgtgcctacccgagtccaagaccccgtaaccgccaccctctcaaaatc
 5 cggaaacacattccggccccgaggtccacctgctgccgccgccgtcgaggagctggccctgaacgagctgggtgacgtgacgtg
 cctggcacgtggcttcagccccaaaggatgtgctggttcgctggctgcaggggtcacaggagctccccgcgagaagtacctgactt
 gggcatcccgaggagcccagccagggcaccaccaccttcgctgtgaccagcactgcgcgtggcagccgaggactggaag
 aagggggacaccttctctgcatggtgggccacgagggcctgccgctggccttcacacagaagaccatcgaccgcttggcgggta
 aaccacccatgtcaatgtgtctgttgcacggcgagggtggactgataatcaga

Amino acid sequence:

mvstaqlfllllwtggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsrgsgs
 gtqfslkisslqpedsgsyfcqhhsdnptwfgggteleikggggsgggsgggssavqlqsgpeseekpgasvkiskcasgys
 ftgynmnwvkqnnkslewignidpyyggtytnrkfkkatltvdksstaymqlksltsedsavyycarsvgpmdywgqg
 15 tsvtvssdqvpstpptpspstpspschprslhrpaled

85. HD37 VL

Nucleotide sequence:

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctcctgggtccaggctccactggtgacattgtctgacct
 20 aatctccagcttcttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatagtt
 atttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatccaccaggt
 ttagtggcagtggtctgggacagacttcacctcaacatccatcctgtggagaagggtggatgctgcaacctatcactgtcagcaaa
 tactgagatccgtggacgttcggtggaggcaccaagctggaaatcaaagggtggcgggtggtcgggcgggtggtgggtcgggtgg
 25 cggcgggagctcg

Amino acid sequence:

metdtlllwlwllwvpgstgdivltqspaslavlsgqratiskasqsvdydgdslwnwyqqipgqppklliidasnlvsgipprf
 sgsrgsgtdflnihpvekvaatyhcqqstedpwtfgggtkleikgggsgggsgggsgss

86. HD37 VH

Nucleotide sequence:

gggagctcgcaggttcagctgcagcagctctggggctgagctggtagggcctgggtcctcagtgaaagatttctgcaaggcttctggc
 tatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggctcttgagtggattggacagatttggcctggagat
 35 ggtgataactaactaatggaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaactcagc
 agcctagcatctgaggactctgcggctatttctgtcaagacgggagactacgacggtaggccgttattactatgctatggactactg
 ggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:

(gss)qvqlqqsgaelvrpgssvkiskcasgyafssywmnwwkqrpqgglewigqiwpdgdtnyngkfkgkatltadesss
 40 taymqlsslasedsavfyfcarrettvgyryyamdywgqgtsvtvssdq

87. HD37 scFv

Nucleotide sequence:

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctcctgggtccaggctccactggtgacattgtctgacct
 45 aatctccagcttcttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatagtt

atttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccacccaggt
 ttagtggcagtggtgctgggacagacttcaccctcaacatccatcctgtggagaagggtggatgctgcaacctatcactgtcagcaaa
 tactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaagggtggcgggtggctcgggcgggtggtgggtcgggtg
 5 cggcggatcgtcagaggttcagctgcagcagctctggggctgagctggtgaggcctgggtcctcagtgaaagattcctgcaaggctc
 tggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtggttgacagatttggcctgg
 agatggtgataactacaatgaaagttcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaact
 cagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggact
 actggggtcaaggaacctcagtcaccgtctcctctgatcag

10 Amino acid sequence:
 metdtlllwlwllwvpgstgdivltqspaslavslgqratisckasqsvdydgdssylwyyqqipgppklliidasnlvsgipprf
 sgsgsgtdftlnihpvekvdadyhcqqstedpwtfgggtkleikgggsgggsgggssqvqlqqsgaelvprgssvkisck
 asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadessstaymqlsslasedsavyfcarrettvgr
 yyyamdywgqgtsvtvssdq

15

88. HD37 VHL11S:

Nucleotide sequence:

gggagctcgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgaaagattcctgcaaggcttctggc
 tatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtggttgacagatttggcctggagat
 20 ggtgataactacaatgaaagttcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaactcagc
 agcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggactactg
 gggtaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:

25 (gss)qvqlqqsgaesvprgssvkisckasgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadess
 staymqlsslasedsavyfcarrettvgryyyamdywgqgtsvtvssdq

89. HD37 scFv VHL11S:

Nucleotide sequence:

30 Aagcttggcccatggagacagacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgctgacc
 caatctccagcttcttggctgtgtcttagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatag
 tatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccacccagg
 ttagtggcagtggtgctgggacagacttcaccctcaacatccatcctgtggagaagggtggatgctgcaacctatcactgtcagcaaa
 gtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaagggtggcgggtggctcgggcgggtggtgggtcgggtg
 35 gcggcgggagctcgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgaaagattcctgcaaggct
 tctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtggttgacagatttggcctg
 gagatggtgataactacaatgaaagttcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaac
 tcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggac
 tactggggtcaaggaacctcagtcaccgtctcctctgatcag

40

Amino acid sequence:

metdtlllwlwllwvpgstgdivltqspaslavslgqratisckasqsvdydgdssylwyyqqipgppklliidasnlvsgipprf
 sgsgsgtdftlnihpvekvdadyhcqqstedpwtfgggtkleikgggsgggsgggssqvqlqqsgaesvprgssvkisck
 asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadessstaymqlsslasedsavyfcarrettvgr
 45 yyyamdywgqgtsvtvssdq

90. HD37 scFv IgAH hIgG1 WCH2 T4CH3

Nucleotide sequence

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggttcaggctccactggtgacattgtgtgaccc
aatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatagtt
5 atttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccaccaggt
ttagtggcagtggtctgggacagacttcacctcaacatccatcctgtggagaaggtggatgctgcaacctatcactgtcagcaaaag
tactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcggtggctcgggcggtggtgggtcgggtgg
cggcgggagctcgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgaagatttctgcaaggctt
ctggctatgattcagtagctactggatgaactgggtgaagcagaggcctggacagggcttgagtggattggacagatttggcctg
10 gagatggtgataactacaatgaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaac
tcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggac
tactggggtcaaggaacctcagtcaccgtctcctctgatcagccagtccctcaactccacctaccccatctccctcaactccacctac
cccatctccctcatgctgccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcac
gtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacggcctcaagtgggaagagcgctgttcaaggaccac
15 ctgaccgtgacctctgtggctgtacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcactt
gactgtgcctaccccgagtcgaagaccccgtaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctg
ctgccgccgctcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgc
tggttcgctggctgcagggttcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccaggggca
ccaccacctcgtgtgaccagcactgctgcgctggcagccgaggactggaagaagggggacaccttctcctgcatggtgggcca
20 cgaggccctgccgtggccttcacacagaagaccatcaccgcttggcgggtaaaccacccatgtcaatgtgtctgttgcacatggc
ggaggtggactgataatctaga

Amino acid sequence

metdtlllwlwllwvpgstgdivltqspaslavslgqratisckasqsvdydgdssylnwyqqipgppklliidasnlvsgipprf
25 sgsrgstgdtflnihpvekvdadyhcqqstedpwtfgggtkleikgggsgggsgggssqvqlqqsgaesvrpgssvkisk
asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgatltadesstaymqlsslasedsavfyfcarrettvgr
yyyamdywgqgtsvtvssdqpvpstptpsptpspcchprlsrlhrpaledlllgseailtctltglrdasgvtftwtpssgks
avqgppdrldlcgysvssvlpgaepwnhgtftctaaypesktpltatlsksntfrpevhllpppseelalnelvtlclargfsp
kdvlvrwlqgsqelprekyltwasrqepsqgtttfavtsilrvaaedwkkgdtfscmvghealplftqktidrlagkpthvnsv
30 vmaevd

91. HD37 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggttcaggctccactggtgacattgtgtgaccc
35 aatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatagtt
atttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccaccaggt
ttagtggcagtggtctgggacagacttcacctcaacatccatcctgtggagaaggtggatgctgcaacctatcactgtcagcaaaag
tactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcggtggctcgggcggtggtgggtcgggtgg
cggcggatcgtcacaggttcagctgcagcagctctggggctgagctggtgaggcctgggtcctcagtgaagatttctgcaaggcttc
40 tggctatgattcagtagctactggatgaactgggtgaagcagaggcctggacagggcttgagtggattggacagatttggcctgg
agatggtgataactacaatgaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaact
cagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggact
actggggtcaaggaacctcagtcaccgtctcctctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtcctcagc
acctgaactcctgggtggaccgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccggaccctgaggtcaca
45 tgcgtgggtgggtggacgtgagccacgaagacctgaggtcaagttaactgttacgtggacggcgtggaggtgcataatgccaaaga
caaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaa

ggagtacaagtgcaaggtctccaacaaagccctcccagcccccacgagaaaaccatctccaaagccaaagggcagccccgaga
 accacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatc
 caagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgac
 ggctccttcttctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgttctcatgctccgtgatgcatgag
 5 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

metdtlllwlwllwvpgstgdivltqspaslavslgqratiskasqsvdydgdslwnwyqqipgppklliidasnlvsgipprf
 sgsgsgtdflnihpvekvdadyhcqqstedpwtfgggtkleikggggsgggsgggssqvlqqsgaelvrpgssvkisk
 10 asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadessstaymqlsslasedsavfcarrettvgr
 yyyamdywqgtsvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnw
 yvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltk
 nqvsltlvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqksls
 pgk

15

92. HD37 scFv VH L11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgccgcatggagacagacacactcctgctatgggtgctgctgcttgggtccaggtccactggtgacattgtgctgacct
 aatctccagcttcttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatagt
 20 atttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcacaaatctagtttctgggatcccaccaggt
 ttagtggcagtggtctgggacagacttaccctcaacatccatcctgtggagaaggtggatgctgcaacctatcactgtcagcaaa
 tactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcgggtgctcgggcgggtggtgggtcgggtg
 cggcgggagctcgcaggttcagctgcagcagcttggggctgagtcggtgaggcctgggtcctcagtgaagatttctgcaaggctt
 ctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggctttagtgattggacagatttggcctg
 25 gagatggtgataactacaatggaaagtcaagggtaaagccactctgactgcagacgaatctccagcagcctacatgcaac
 tcagcagcctagcatctgaggactctgcggtctatttctgtcaagacgggagactacgacggtagggcgttattactatgctatggac
 tactgggtgcaaggaacctcagtcaccgtctcctctgacagagcccaaatcttctgacaaaactcacacatccccaccgtcctcag
 cacctgaactcctggggggaccgtcagcttcttcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtca
 catgcgtggtggtggacgtgagccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgcaa
 30 gacaaagccgcgggaggagcagtacaacagcagctaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatgg
 caaggagtacaagtcaaggtctccaacaaagccctcccagcccccacgagaaaacaatctccaaagccaaagggcagccccg
 agaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttct
 atccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccg
 acggctccttcttctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgttctcatgctccgtgatgcatg
 35 aggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

metdtlllwlwllwvpgstgdivltqspaslavslgqratiskasqsvdydgdslwnwyqqipgppklliidasnlvsgipprf
 sgsgsgtdflnihpvekvdadyhcqqstedpwtfgggtkleikggggsgggsgggssqvlqqsgaelvrpgssvkisk
 40 asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadessstaymqlsslasedsavfcarrettvgr
 yyyamdywqgtsvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnw
 yvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltk
 nqvsltlvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqksls
 pgk

45

91. L6 VL

Nucleotide sequence:

atggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctcccagctctccagc
aatcctgtctgcatctccaggggagaagggtcacattgacttgaggccagctcaagtgttaagttcatgaactggtaggcagcagaag
ccaggatcctccccaaaccctggatttatgccacatccaatttggcttctgagttccctggcgttcagtggcgagtggtctgggacc
5 tcttactctctcgaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagtaaccactcacgttcgg
tgctgggaccaagctggagctgaaagagctctctggtggcgggtggctcggcggtggtgggtcgggtggcggcggtatct

Amino acid sequence:

10 Mdfqvqifsflisasvimrsgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwiysnlasefpgrfsge
wsgtsylaisrveadaatyycqqwnsnpltfagtklelkelsgggsgggsgggsgggsg

92. L6 VH

Nucleotide sequence:

15 cagatccagttggtgcagcttgacactgagctgaagaagcctggagagacagtcagatctcctgcaaggcttctgggtataccttca
caaatatggaatgaactgggtgaagcaggctccaggaaagggttaagtggatgggctggataaacactacactggacagcca
acatagctgatgactcaaggacggttgccttctcttggaaacctctgcctacactgcctatttgagatcaacaacctcaaaatg
aggacatggctacatattctgtgcaagatttagctatgtaactcacgttacgctgactactggggccaaggcaccactctcacagctc
cctctgatca

20 Amino acid sequence:

qqlvqsgpelkpkpgetvkiskcasgytftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqi
nnlknedmatyfcarsygnryadywgqgttlvtssd

93. L6 scFv

25 Nucleotide sequence:

atggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctcccagctctccagc
aatcctgtctgcatctccaggggagaagggtcacattgacttgaggccagctcaagtgttaagttcatgaactggtaggcagcagaag
ccaggatcctccccaaaccctggatttatgccacatccaatttggcttctgagttccctggcgttcagtggcgagtggtctgggacc
tcttactctctcgaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagtaaccactcacgttcgg
30 tgctgggaccaagctggagctgaaagagctctctggtggcgggtggctcggcggtggtgggtcgggtggcggcggtatctctgcag
atccagttggtgcagcttgacactgagctgaagaagcctggagagacagtcagatctcctgcaaggcttctgggtataccttcacaa
actatggaatgaactgggtgaagcagggtccaggaaagggttaagtggatgggctggataaacactacactggacagccaaca
tatgctgatgactcaaggacggttgccttctcttggaaacctctgcctacactgcctatttgagatcaacaacctcaaaatgagg
acatggctacatattctgtgcaagatttagctatgtaactcacgttacgctgactactggggccaaggcaccactctcacagctcct
35 ctgatca

Amino acid sequence:

40 mdfqvqifsflisasvimrsgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwiysnlasefpgrfsge
wsgtsylaisrveadaatyycqqwnsnpltfagtklelkelsgggsgggsgggsglqqlvqsgpelkpkpgetvkiskca
sgytftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqinnlknedmatyfcarsygnry
adywgqgttlvtssd

94. L6 VHL11S

Nucleotide sequence;

45 ctgcagatccagttggtgcagcttgacactgagctgaagaagcctggagagacagtcagatctcctgcaaggcttctgggtatacct
tcacaaactatggaatgaactgggtgaagcagggtccaggaaagggttaagtggatgggctggataaacactacactggacag

ccaacatatgctgatgacttcaaggacggtttgccttctctttggaacctctgcctacactgcctatttgcagatcaacaacctcaaaa
atgaggacatggctacatatctgtgcaagatttagctatggttaactcacgttacgctgactactggggccaaggcaccactctcaca
gtctcctctgatca

- 5 Amino acid sequence:
qiqlvqsgpeskpkpgetvkiskasgytftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqi
nnlknedmatyfcarsygnrsryadywgqgttlvtssd

10 95. L6 VH L11S scFv

Nucleotide sequence;

Aagcttggttatggatttcaagtgcagatttgcagcttctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctcc
cagctctccagcaatctgtctgcattctccaggggagaagggtcacattgacttgcagggccagctcaagtgttaagttcatgaactggta
ccagcagaagccaggatcctccccaaacctggatttatgccacatccaatttgcttctgagttccctggctcgttcagtggtcagtg
15 ggtctgggacctcttactctctcgcaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagtaaccc
actcacgttcggtgctgggaccaagctggagctgaaagagctctctgggtggcgggtggctcgggcgggtgggtgggtcgggtggcggc
ggatctctgcagatccagttggtgcagctctggacctgagtcgaagaagcctggagagacagtcagatctctgcaaggcttctggg
tataccttcacaaactatggaatgaactgggtgaagcaggctccaggaaaggggttaagtggatgggctggataaacacctacact
ggacagccaacatatgctgatgacttcaaggacggtttgccttctctttggaacctctgcctacactgcctatttgcagatcaacaac
20 ctcaaaaatgaggacatggctacatatctgtgcaagatttagctatggttaactcacgttacgctgactactggggccaaggcaccac
tctcacagctctctctgatca

Amino acid sequence:

- mdfqvqifsfllisasvmsrgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwiysnlasefpgrfsge
25 wsgtsyslaisrveadaatyycqwnsnpltfagtklelkelsgggsgggsgggslqiqlvqsgpeskpkpgetvkiskca
sgyftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqinnlknedmatyfcarsygnrsry
adywgqgttlvtssd

30 96. L6 Or L6 VHL11S scFv IgAH hIgG1 WCH2 WCH3

Nucleotide sequence: (L6 VHL11S is shown)

aagcttggttatggatttcaagtgcagatttgcagcttctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctccc
agtctccagcaatctgtctgcattctccaggggagaagggtcacattgacttgcagggccagctcaagtgttaagttcatgaactggta
35 cagcagaagccaggatcctccccaaacctggatttatgccacatccaatttgcttctgagttccctggctcgttcagtggtcagtg
gtctgggacctcttactctctcgcaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagtaaccca
ctcacgttcggtgctgggaccaagctggagctgaaagagctctctgggtggcgggtggctcgggcgggtgggtgggtcgggtggcggc
gatctctgcagatccagttggtgcagctctggacctgagtcgaagaagcctggagagacagtcagatctctgcaaggcttctgggt
ataccttcacaaactatggaatgaactgggtgaagcaggctccaggaaaggggttaagtggatgggctggataaacacctacactg
40 gacagccaacatatgctgatgacttcaaggacggtttgccttctctttggaacctctgcctacactgcctatttgcagatcaacaacct
caaaaatgaggacatggctacatatctgtgcaagatttagctatggttaactcacgttacgctgactactggggccaaggcaccactc
tcacagctctctctgatcagcagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgcgcacctg
aactctgggggggaccgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccggaccttgaggtcacatgcg
tggtgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaa
45 gccgcgggaggagcagtaaacagcagctaccgtgtgggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggga
gtacaagtgaaggctctccaacaaagccctcccagccccatcgagaaaaacaatctcaaaagccaaagggcagccccgagaacc

acaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatccca
gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggct
ccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctct
gcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

5

Amino acid sequence:

mdfqvqifsfllisasvmsrgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwiatsnlasefpgrfsge
wsgtsylaisrveaadaatyycqqwnsnpltfagatklelkelsgggsgggsgggslqqlvqsgpeskpkpetvkiscka
sgyftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqinnlknedmatyfcarsfygnsry
adywgqgttlvtssdqpvstptpspstpspscapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnw
yvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltk
nqvsltlvkgyfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksls
pgk

10

15

97. L6 scFv VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgttgttatggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggacaaattgttctctccc
agtctccagcaatcctgtctgcattctccaggggagaaggtcacattgacttgcaggccagctcaagtgttaagttcatgaactggtag
cagcagaagccagatcctccccaaaccctggatttatgccacatccaatttggttctgagttccctggcgttcagtgccgagtg
gtctgggaccttactctctcgaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagtaaccca
ctcacgttcggtgctgggaccaagctggagctgaaagagctctctggtggcggtggctcggcggtggtgggtcgggtggcgggc
gatctctgcagatccagttggtgcagcttgacgtgagcgaagaagcctggagagacagtcagatctcctgcaaggcttctgggt
ataccttcacaaactatggaatgaactgggtgaagcaggtccaggaaaggggttaagtggatgggctggataaacacctacactg
gacagccaacatatgctgatgacttcaagggacggtttgccttctctttgaaacctctgcctacactgcctatttgcagatcaacaacct
caaaaatgaggacatggctacataattctgtgcaagatttagctatgtaactcacgttacgctgactactggggccaaggcaccactc
tcacagtctcctctgatcaggagcccaaatctctgacaaaactcacatccccaccgtcctcagcacctgaactcctggggggacc
gtcagtcttctcttcccccaaaacccaaggacacctcatgatctccggacctgaggtcacatgcgtggtggtggacgtgag
ccacgaagacctgagggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
gtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtct
ccaacaaagccctcccagccccatcgagaaaacaatcctcaaagccaaagggcagccccgagaaccacaggtgtacacctgc
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtggag
tgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaa
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

35

Amino acid sequence:

mdfqvqifsfllisasvmsrgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwiatsnlasefpgrfsge
wsgtsylaisrveaadaatyycqqwnsnpltfagatklelkelsgggsgggsgggslqqlvqsgpeskpkpetvkiscka
sgyftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqinnlknedmatyfcarsfygnsry
adywgqgttlvtssdqpkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltknqvsltc
lvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksls
lspgk

40

45

98. P238 CH2

Nucleotide sequence:

accgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaa
caatctccaaagccaaa

Amino acid sequence:

5 pellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdw
l
ngkeykckvsnkalpapietiskak

99. P238 CH2 WCH3

a. P238S CH2 WCH3

10 Nucleotide sequence:

cctgaactcctgggggatcgctcagttcttcttcccccaaaaccaaggacaccctcatgatctccggaccctgaggtcacat
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtgtcagcgtcctaccgtcctgcaccaggactggctgaatggcaa
ggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgaga
15 accacaggtgtacaccctgccccatcccggtgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatc
ccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgac
ggctccttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

20

Amino acid sequence:

pellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdw
l
ngkeykckvsnkalpapietiskakgqprepvytlppsrdeitknqvsitclvkgfypsdiavewesngqpennyktppvl
dsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslslspgk

25

b. (SSS-S)H P238S CH2 WCH3

Nucleotide sequence:

tgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctggggggatcgctcagttctctt
30 tcccccaaaaccaaggacaccctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccct
gaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtgtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccct
cccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccgga
tgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtggagtgaggagcaatg
35 ggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttctctctacagcaagctcaccgtggac
aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc
cctgtctccgggtaaatgatctaga

Amino acid sequence:

40 dqepkssdkthtspssapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeq
ynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeitknqvsitclvkgfypsdiavew
esngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslslspgk*

45 100. a.CD16-6 low (ED) +NL+ (SSS-S)H P238S CH2 WCH3

Nucleotide sequence:

ggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggatcgtcagcttctcttcccc
 caaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtagcgtgagccacgaagacctgaggt
 caagttcaactggtacgtggacggcgtggaggtgcataatgccaaagccgcgggaggagcagtacaacagcacgtaccg
 tgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggtctccaacaaagccctccag
 5 ccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggtgagc
 tgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggca
 gccggagaacaactacaagaccacgcctcccgctggtgactccgacggctccttctctctacagcaagctcaccgtggacaaga
 gcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgt
 cccgggtaaatgatctaga

Amino acid sequence:

mwqlllptallllvsagmrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvddsg
 eyrcqtnlstlspvqlvhighwlllqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyipkatlkdsqsyf
 crglvgsknvssetvnititqgladqepkssdkthtspssapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkf
 15 nwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdel
 tknqvsltlvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqksls
 lspgk

101. b.CD16-6 low (ED)+HE4LP+(SSS-S)H P238S CH2 WCH3

Nucleotide sequence:

aagcttccgccaatgcctgctgtgccttaggccccgtagccgccccctcctcctcagcctgctgctgttcggcttcaccctagtctc
 aggcaccgggtgcaatcgggactgaagatctccaaaggctgtggtgttctggagcccaatggtacagggtgctcagaaggaca
 gtgtgactctgaagtgccaggaggcctactcccctgaggacaattccacacagtggttcacaatgagagcctcatctcaagccagg
 cctcgagctacttcattgacgtgccacagtcgacgacagtggagagtacaggtgccagacaaacctctccacctcagtaccgg
 25 gtgcagctagaagtccatcctggtggtgtgtgctccaggccccctgggtgggtgttcaaggaggaagacctattcacctgaggtgtc
 acagctggaagaacactgctctgcataaggtcacatatattacagaatggcaaaggcaggaagtattttcatcataattctgacttctacat
 tccaaaagccacactcaagacagcggctcctactctgcagggggctgtgtggagtaaaaatgtgtcttcagagactgtgaacatc
 accatcactcaaggtttggctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgg
 ggggatcgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtgg
 30 acgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcggg
 aggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagt
 caaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgta
 caccctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggcttctatccagcgacatcg
 ccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccgtgctggactccgacggctccttctcct
 35 ctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacc
 actacacgcagaagagcctctccctgtctccgggtaaatgatctagaaa

Amino acid sequence:

mpacrlgplaaalllllfgftlvsagmrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassy
 40 fidaatvddsgeyrcqtnlstlspvqlvhighwlllqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyi
 pkatlkdsqsyfcrglvgsknvssetvnititqgladqepkssdkthtspssapellggssvflfppkpkdtlmsrtpevtcvvv
 dvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgppre
 pqvytlppsrdeltnqvsltlvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmh
 ealnhhtqkslsispgk

102. CD16-9 high (ED) (SSS-S)H P238S CH2 CH3

a. CD16-9 high (ED)NL+(SSS-S)H P238S CH2 CH3

Nucleotide sequence:

gttgtaagcttgccgcatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgaggactgaagatctccca
5 aaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggacagtgtagctctgaagtgccagggagcctactcccc
gaggacaattccacacagtggttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtcgacg
acagtgagagtagagtgccagacaaacctctccacctcagtgacccgggtgcagctagaagtccatcggctggctgttgctcc
aggccctcggtgggtgttcaaggaggaagaccctattcacctgaggtgtcacagctggaagaacactgctctgcataaggtcacat
10 attacagaatggcaaaggcaggaagtatttcatcataattctgacttctacattccaaaagccacactcaaagacagcggctcctactt
ctgcagggggcttttgggagtaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggctgatcaggagcccaaat
cttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggatcgtcagttcttcttccccccaaaaccaa
ggacacctcatgatctccggaccctgaggtcacatgcgtgggtgggtggacgtgagccacgaagaccctgaggtcaagttaact
ggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagc
gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcga
15 gaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaa
ccaggtcagcctgacctgctgtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaac
aactacaagaccacgctcccgctggtgactccgacggctccttcttctctacagcaagtcaccgtggacaagagcaggtggca
gcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaa
20 atgatctagaaa

Amino acid sequence:

mwqlllptallllvsagmrtdlpkavvflepqwyrlekdsvtlkcgayspednstqwfhnslissqassyfidaatvddsg
eyrcqtnlstldpvlqlevhigwllqaprwwfkeedpihlrhwkntalhkvtlylqngkgrkyfhnsdfyipkatlkdsqsyf
25 crglfgsknvsssetvntitqgladqepkssdkthtspssapellggssvflfppkpkdtlmisrtpevtcvvvdvshedpevkfn
wyvdgvevhnatkpreeqnystyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeit
knqvsltlvkgfypsdiavewesngqpennyktppvldsdgsfflyskltvdkswqqgnvfscsvmhleahnhytqksls
spgk

CD16-9 high (ED)+HE4LP+hIgG1 (SSS-S)H P238S CH2 CH3

Nucleotide sequence:

aagcttgccgcatgctgtgctgcttaggcccgtagccgccgcccctcctcctcagcctgctgctgttcggcttcaccctagtctc
aggcaccgggtgcaatgaggactgaagatctcccaaaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggaca
gtgtgactctgaagtccagggagcctactccctgaggacaattccacacagtggttcacaatgagagcctcatctcaagccagg
cctcagctacttcattgacgctgccacagtcgacgacagtgagagtagcaggtgccagacaaacctctccacctcagtgacccg
35 gtgcagctagaagtccatcgcgctggctgtgtgctccaggccccctgggtgggtgttcaaggaggaagaccctattcacctgaggtgc
acagctggaagaacactgctctgcataagggtcacatatttacagaatggcaaaggcaggaagtatttcatcataattctgacttctacat
tccaaaagccacactcaaagacagcggctcctacttctgcagggggcttttgggagtaaaaatgtgtcttcagagactgtgaacatca
ccatcactcaaggtttggctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggg
gggatcgtcagcttcttcttccccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtgggtgga
40 cgtgagccacgaagaccctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcggga
ggagcagtacaacagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgc
aaggtctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtac
acctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaggcttctatccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctggtgactccgacggctccttcttctct
45 acagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacca
ctacacgcagaagagcctctcctgtctccgggttaaatgatctagaaa

Amino acid sequence:

mpacrlgplaaallslfllvsgtgamrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassy
fidaatvddsgeyrcqtnlstlsvpqlvhighwillqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyi
5 pkatlkdsysyfcrglvgsknvssetvnititqgladqepkssdkthtspssapellggssvflfppkpkdtlmisrtpvctvvvd
vshedpevkfnwyvdgvevhnaktkpreeqynstyrsvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprep
qvtylppsrdeltknqvsltlvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhe
alhnhytqkslslspgk

10

103. a. CD16 ED low (native leader)

Nucleotide sequence:

aagcttgccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgcggactgaagatctcccaaaggc
tgtggtgttctggagcctcaatggtacaggggtctcgagaaggacagtgtgactctgaagtgccaggagcctactccctgagga
15 caattccacacagtgtttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtcgacgacagt
ggagagtacaggtgccagacaaacctctccacctcagtgaccgggtgcagctagaagtccatcggctggctgttgctccaggc
ccctcggtgggtgttcaaggaggaagaccctattcacctgaggtgtcacagctggaagaacactgctctgcataaggtcacatatta
cagaatggcaaaggcaggaagtattttcatcataattctgacttctacattccaaaagccacactcaaagacagcggctcctacttctgc
aggggggctgttgggagtaaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggttggctgatcaaaa

20

Amino acid sequence:

mwqlllptallllvsagmrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvddsg
eyrcqtnlstlsvpqlvhighwillqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyipkatlkdsysyf
25 crglvgsknvssetvnititqgladq

25

b. CD16 ED low (HE4 leader)

Nucleotide sequence

aagcttgccgccatgcctgctgtgcctagggcccgctagccgccgccctcctcctcagcctgctgctgttcggcttcaccctagtctc
aggcaccgggtgcaatgcggactgaagatctcccaaaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggaca
30 gtgtgactctgaagtgccaggagcctactccctgaggacaattccacacagtgtgttcacaatgagagcctcatctcaagccagg
cctcgagctacttcattgacgctgccacagtcgacgacagtggagagtacaggtgccagacaaacctctccacctcagtgaccgg
gtgcagctagaagtccatcctcggtggctgttgctccaggccctcgggtgggtgttcaaggaggaagaccctattcacctgaggtgtc
acagctggaagaacactgctctgcataaggtcacatatttacagaatggcaaaggcaggaagtattttcatcataattctgacttctacat
tccaaaagccacactcaaagacagcggctcctacttctgcaggggggctgttgggagtaaaaatgtgtcttcagagactgtgaacatc
35 accatcactcaaggttggctgatcaaaa

Amino acid sequence

mpacrlgplaaallslfllvsgtgamrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassy
40 fidaatvddsgeyrcqtnlstlsvpqlvhighwillqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyi
pkatlkdsysyfcrglvsknvssetvnititqgladq

104. a. CD16 ED high (native leader)

Nucleotide sequence:

45

gttgtaagcttgccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatcgggactgaagatcctcca
aaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggacagtgactctgaagtggcagggagcctactccct
gaggacaattccacacagtgtttcacaatgagagcctcatctcaagccaggcctcagctacttcattgacgctgccacagtcgacg
acagtggagagtacaggtgccagacaaacctctccacctcagtgacctgggtgcagctagaagtccatacggctggctgttgctcc
5 agggccctcgggtgggtgttcaaggaggaagacctattcacctgaggtgtcacagctggaagaacactgctctgcataaggtcacat
atttacagaatggcaaaggcaggaagtattttcatcataattctgacttctacattccaaaaggccacactcaaagacagcggctcctactt
ctgcagggggcttttgggagtaaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggctgatcaa

Amino acid sequence:

10 mwqlllptalllvsagmrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvddsg
eyrcqtnlstlsvpqlvhighwlllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyipkatlkdsqsyf
crglfgsknvssetvnititqgladq

b. CD16 ED high (HE4 leader)

15 Nucleotide sequence:

aagcttgccgccatgcctgctgtgcctaggcccgtagccgccgcccctcctcagcctgctgctgttcggcttcaccctagtctc
aggcaccgggtgcaatcgggactgaagatcctccaaaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggaca
gtgtgactctgaagtccagggagcctactccctgaggacaattccacacagtgtttcacaatgagagcctcatctcaagccagg
cctcagctacttcattgacgctgccacagtcgacgacagtgagagtagcaggtgccagacaaacctctccacctcagtgacctcg
20 gtgcagctagaagtccatacggctggctgtgtcctcaggccctcgggtgggtgttcaaggaggaagacctattcacctgaggtgtc
acagctggaagaacactgctctgcataaggtcacatatattacagaatggcaaaggcaggaagtattttcatcataattctgacttctacat
tccaaaaggccacactcaaagacagcggctcctacttctgcagggggcttttgggagtaaaaatgtgtcttcagagactgtgaacatca
ccatcactcaagggttggctgatcaa

25 Amino acid sequence:

mpacrllgplaaallslfllvsgtgamrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassy
fidaatvddsgeyrcqtnlstlsvpqlvhighwlllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyi
pkatlkdsqsyfcrglfgsknvssetvnititqgladq

30

105. 2e12 scFv (SSS-S)H P238S CH2 WCH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca
atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaagtgtgaatattatgcacaagtta
35 atgcagtggtagcaaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctgggtccctgccagg
tttagtggcagtggtgtgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattttgtcagcaagt
aggaaggttcttgacgttcgggtggaggcaccaagctggaaatcaaacgggtggcggtggctcgggcggaggtgggtcgggt
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctgggtggcggcctcacagagcctgtccatcacatgcaccgtct
caggggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatgggtg
40 atggaagcacagactataattcagctctcaaatccagactgagcatccaaggaactccaagagccaagtttcttaaaaatgaa
cagctctgaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
tggggggatcgtcagcttctccttccccccaaaacccaaggacacctcatgatctccgggaccttgaggtcacatgcgtgggtggt
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
45 ggaggagcagtagaacagcaggtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag
tgcaaggtctccaacaaagccctccagccccatcgagaaaacctctcaaaagccaaagggcagccccgagaaccacaggtgt

acaccctgcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcccagcgacatc
 gccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
 cactacacgcagaagagcctctccctgtctccgggtaaaaggatccttcgaacctgctcccatcctggccattacctaatactcagt
 5 aaatggaattttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaaggga
 aagtgtacgccctgtataaatcgat

Amino acid sequence:

mdfqvqifsflisasvmsrgvdivltqspaslavslgqratiscrasesveyvtslmqwyqqkpgqppkllisaasnvesgvp
 10 arfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggtkleikrgggsgggsgggsgggsgqvqlkesgpglvapsqslsit
 ctvsgfsltygvnwvrqppgkglewlgmiwgdgstdynsalksrslsitkdnksqvflkmnslqtdtdaryycardgysnfh
 yyvmdywgqgtsvtvssdlepkssdkthtspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy
 vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeitkn
 qvsltlvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp
 15 gkadpsnllpswaitlisvngifviccltycfaprerrrrmerlrresvrpv

106. 10A8 scFv (SSS-S)H P238SCH2 WCH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacac
 20 agtctccatcctcactgtctgcatctctgggaggcaaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttg
 taccaacacaagcctggaaaagggtcccagggtgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtggaa
 gtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataatcttcc
 attgacgttcggctcggggacaaagtggaaataaaacgggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcgatct
 gatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtcctctgtctcctcactgctctgtcactggctactccatcacc
 25 agtggtttctactggaactggatccgacagttccgggaacaaactggaatggatgggccacataagccacgacggtaggaataac
 tacaacctatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtgactactgagga
 cacagctacatatttctgtgcaagacactacgtagtagcggagctatggactactggggtcaaggaacctcagtcaccgtctcctct
 gatctggagcccaaatcttctgacaaaactacacatccccaccgtccccagcacctgaactcctggggggatcgtcagtccttctctt
 cccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtgggtgggtgacgtgagccacgaagacct
 30 gaggtcaagttcaactgtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
 taccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccct
 ccagcccccatcgaaaaacatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggga
 tgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg
 ggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggac
 35 aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc
 cctgtctccgggtaaaagcggatccttgaacctgctcccatcctgggccattacctaatactcagtaaatggaattttgtgatatgctgc
 ctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcga
 t

40 Amino acid sequence:

mdfqvqifsflisasvmsrgvdiqmtqspsslsaslggkvttitcasqdikkyigwyqhkpkgprlliiyytstlqpgipsrfs
 sgsgrdyslsimlepediatyycqydnpltfsgstkleikrgggsgggsgggsgggsgdvqlqesgpglvkpsqslsltsvtygs
 itsgyfwnwirqfpgnklewmghishdgrnynpslinrisitrdtsknqfflklssvttdtatyfcarhygssgamdywgqgt
 svtvssdlepkssdkthtspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktk
 45 preeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeitknqvsltlvkgfyps

diavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslspsgkadsnllpswa
itlisvngifviccltycfaprcrerrrnerlrresvrpv

5 **107. 40.2.36 scFv (SSS-P)H P238SCH2 WCH3-hCD80TM/CT**

Nucleotide sequence:

Amino acid sequence:

10

108. 2H7 scFv VHL11S (SSS-P)H P238SCH2CH3-hCD80TM/CT

Nucleotide sequence:

15

20

25

30

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaattacatgcactgg
taccagcagaagccagatcctccccaaacctggatttatgccccatccaacctggcttctggagctccctgctcgttcagtgga
gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcgggtctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggcttatctacagcagctctggggctgagtcggtagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggatggagctatttatccaggaaatggtgatact
tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
atctgaagactctcggtctatttctgtgcaagagtgggtactatagtaacttactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatctggagcccaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga
tcgtcagcttctcttcccccaaaaccaaggacacctcatgatctccggaccttgaggtcacatgcgtggtggtggacgtga
gccacgaagacctgaggtcaagtcaactgtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc
agtacaacagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtc
tccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtacacctg
ccccatcccggtgagctgaccaagaaccaggtcagctgacctgctgctgtaaaggcttctatcccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacg
cagaagagcctctccctgtctccgggtaaagcggatccttgaacctgctccatcctgggccattaccttaattcagtaaatggaatt
tttgtgatatgctgcctgacctactgcttggcccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgc
cctgtataaatcgat

Amino acid sequence:

35

40

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypslnlasgvparfsg
sgsgtsysltisrveadaatyycqqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas
gytftsynmhwwkqtrqglewigaiypngdtsynqkfkkatltvdksstaymqsltsedsavvfcarvvyysnsywy
fdvwgtgtvtvssdlepkssdkthtspspapellggssvflfpkpkdltmisrtpevtcvvvdvshedpevkfnwyvdgve
vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltknqvsitcl
vkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslspsgkads
nllpswaitlisvngifviccltycfaprcrerrrnerlrresvrpv

109. G19-4 scFv (SSS-P)H P238SCH2 WCH3-hCD80TM/CT

Nucleotide sequence:

45

aagcttatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacac
agactacatctccctgtctgcctctctgggagacagagtcaccatcagttgcagggaagtcaggacattcgcaattattttaaactgg

tatcagcagaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtgccatcaagggttcagtggcag
 tgggtctggaacagattattctcaccattgccaacctgcaaccagaagatattgccacttacttttccaacagggttaacgcttccg
 tggacgttcgggtggaggcaccaaactgtaaccaaacgggagctcgggtggcgggtgctcgggcgggtggtgggtcgggtggcg
 cggatctatcgatgaggtccagctgcaacagctctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggcctctggttac
 5 tcattcactggctacatcgtaactggctgaagcagagccatggaaagaaccttgagtggattggacttattaatccatacaaaaggtctt
 actacctacaaccagaaattcaagggaaggccacattaactgtagacaagtcacccagcacagcctacatggagctcctcagctctg
 acatctgaagactctgcagctctattactgtgcaagatctgggtactatggtgactcggactggtacttcgatgtctggggcgagggac
 cacggtcaccgtctcctctgatctggagcccaaatctctgacaaaactcacacaagcccaccgagcccagcacctgaactcctggg
 gggatcgtcagcttctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggtgga
 10 cgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcggga
 ggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgc
 aaggtctccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtac
 acctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
 cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcctct
 15 acagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacca
 ctacacgcagaagagcctctcctgtctccgggtaagcggatccttgaacctgtcccatcctgggccattaccttaatctcagtaa
 atggaattttgtgatatgctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaaa
 gtgtacgccctgtataatcgatactcgag

20 Amino acid sequence:
 mdfqvqifsfllisasvmsrgvdiqmtqtsslsaslgdrvtiscrasqdirnylnwyqqkpdgtvkllyytsrlhsgvpsrfs
 gsgtdysltianlqpediatyfcqqntlpwtfgggtklvtkrelgggsgggsgggsgggsidevqlqsgpelvkgpasmckas
 gysftgyivnwlkqshgknlewiglinpykglttynqkfkkgkatltvdkssstaymellsitsedsavyycarsgyygdswyf
 dvwgagttvtvssdlepkssdkthtspspapellgssvflfppkpkdtlmsrtpetcvvvdvshedpevkfnwyvdgve
 25 vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltcl
 vkgfypsdiavewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslslspgkads
 nllpswaitlisvngifviccltycfaprerrrrerlrresvrpv

30 110. 2e12 scFv (SSS-P)H WCH2 WCH3-hCD80TM/CT

Nucleotide sequence:
 aagcttatgattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
 atctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta
 atgcagtggtagcaacagaaaccaggacagccacccaaactcctcctctgtgcatccaacgtagaatctggggtccctgccagg
 35 tttagtggcagtggtgtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattctgtcagcaa
 aggaaggttccttgacgttcgggtggaggcaccaagctggaaatcaaacggggtggcgggtggtcgggcggaggtgggtcgggt
 ggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcggcctcacagagcctgtccatcacatgcaccgtct
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatggggtg
 atggaagcacagactataattcagctctcaaatccagactgagcatccaaggacaactccaagagccaagtttcttaaaatgaa
 40 cagctctgcaaaactgatgacacagccagataactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
 tggggggaccgtcagcttctccttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtgg
 tggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
 ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag
 45 tgcaaggtctccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgt
 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc

gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaagcggatccttcgaacctgtcccacatcctgggccattacctaatactcagt
aaatggaattttgtgatatgctgcctgacactactgctttgccccaatgacagagagagaaggaggaatgagagattgagaaggga
5 aagtgtacgccctgtataaatcgat

Amino acid sequence

mdfqvqifsfllisasvimsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
arfsghsgstgdfslnihpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit
10 ctvsgfsltygvnwvrpppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqtdtdaryycardgysnfh
yyvmdywggqtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy
vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeitkn
qvsltlvkgfypsdiavewesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp
gkadpsnllpswaitlisvngifviccltycfaprerrrrerlrresvrpv

15

111. 2e12 scFv IgA WH WCH2 T4 CH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta
20 atgcagtggtagcaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgccagg
tttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
aggaaggttccttgacgttcgggtggaggcaccaagctggaaatcaaacggggtggcgggtgctcgggaggaggtgggtcgggt
ggcggcgatctcaggtgcagctgaaggagtcaggacctggcctgggtggcgcctcacagagcctgtccatcacatgcaccgtct
cagggttctcattaaccggctatggtgtaaactgggtcgcagcctccaggaaagggtctggagtggctgggaatgatatggggtg
25 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtgttttataaaatgaa
cagctctgaaactgatgacacagccagataactactgtgccagagatggtatagtaactttcattactatgttatggactactgggtcaa
ggaacctcagtcaccgtctcctcagatcagccagttccctcaactccacctacccatctcctcaactccacctacccatctcctca
tgctgccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgacc
ggcctgagagatgcctcaggtgtcaccttcacctggacgcctcaagtgggaagagcgctgttcaaggaccacctgacctgacct
30 ctgtggctgtacagcgtgtccagtgctcctgccgggctgtgccgagccatggaacctgggaagaccttcactgtcactgctgcctac
cccagatccaagaccccgtaccgccacctctcaaaatccggaaacacattccggcccagggtccacctgtgccgccggcgt
cggaggagctggccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggt
gcaggggtcacaggagctgccccgcgagaagtacgtgacttgggcatccggcaggagcccagggcaccaccaccttcg
ctgtgaccagcactatgcgctggcagccgaggactggaagaagggggacacctctcctgcatggtgggccacgaggccctgc
35 cgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacctatgcaatgtgtctgttgcacggcgagggtggacg
cggatccttcgaacaacctgctccatcctgggccattaccttaatctcagtaaatggaattttgtgatatgctgcctgacctactgctt
gccccaatgacagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgatac

Amino acid sequence:

mdfqvqifsfllisasvimsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
arfsghsgstgdfslnihpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit
40 ctvsgfsltygvnwvrpppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqtdtdaryycardgysnfh
yyvmdywggqtsvtvssdqpvpstpptpsptpspschprlsllhrpaledlllgseailtctltglrdasgvftwtpssgksa
vqppdrdlcgcysvssvlpgaepwnhgtfctaaypesktpatlsksngtfrpevhllpppseelalnelvtlclargfspk
45 dvlvrwlqsgqlprekyltwasrqpsqgtttfavtsilrvaaedwkkgtfscmvghlealplafktidrlagkpthvnsvv
maevdadpsnllpswaitlisvngifviccltycfaprerrrrerlrresvrpv

112. 2e12scFv IgE WCH2WCH3WCH4-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
5 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaaagtta
atgcagtggtagcaacagaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggccctgccagg
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
aggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggaggaggtgggtcgggt
ggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgcctcacagagcctgtccatcacatgcaccgtct
10 cagggttctcattaaccggctatggtgtaaactgggtcggcagcctccaggaaagggtctggagtggctgggaatgatatggggtg
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctaaaaatgaa
cagctctgaaaactgatgacacagccagataactactgtccagagatggtatagtaactttcattactatgttatggactactgggtgcaa
ggaacctcagtcaccgtctcctcagatcacgtctgctccagggaactcaccggccaccgtgaagatcttacagtcgtcctgcgacg
gcggcgggacacttcccccgaccatccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggag
15 gacgggcaggtcatggacgtggacttgcaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcaccc
tcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaag
tgtgcagattccaacccgagagggtgagcgctacctaagccggccagcccgttcgacctgttcatccgcaagtcgccacgat
cacctgtctggtggtggacctggcaccagcaaggggaccgtgaacctgacctggtcccgggccagtggaagcctgtgaaccac
tccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccacctgccggtgggacccgagactggatcgag
20 ggggagacctaccagtgcagggtgacccacccccacctgccaggggcctcatgcggtccacgaccaagaccagcggcccgcg
tgctgccccggaagtctatgcgtttgcgacggcgagtgccggggagccgggacaagcgaccctcgctgcctgatccagaac
ttcatgcctgaggacatctcgggtgcagtggtgcacaacgaggtgcagctcccgacgcccggcacagcacgacgcagccccgc
aagaccaaggggtccggcttcttcgtctcagccgctggaggtgaccagggccgaatgggagcagaaaagatgagttcatctgccg
tgagtcctatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaagcgagatccttcgaagctcc
25 catcctggggccattaccitaaatctcagtaaatggaattttgtgatatgtgcctgacctactgcttgcccaagatgcagagagagaag
gaggaatgagagattgagaagggaagtgtacgccctgtataaatcgata

Amino acid sequence:

mdfqvqifsflisasvimsvrgdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
30 arfsgsgsgtdfslnihpveeddiameyfcqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit
ctvsgfsltygvnwvrppgkglewlgmiwgdgstdynsalksrslsitkdnksqvflkmnslqtdtdaryycardgysnfh
yyvmdywgqgtsvtvssdhvcsrdftpptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmvdvlstattqeg
elastqseltsqkhwlslrdtytcqvyqgthfedstkkcadsnprgvsaylsrspfdlfrksptitclvdlapskgtvnlwsras
gkpvnhstrkeekqrmgtltvtstlpvgrdwiegetyqcrvthphlpralmrsttktspraapevyafatpewpgsrdrkrtlacli
35 qnfmpedisvqwlhnevqlpdarhsttqprktkgsgffvfrlevtraeweqkdeficravheaaspsqtvqravsvnpkgadp
sklpswaitlisvngifviccltycfaprcrerrrmerlrresvrp

113. mFADD-TM/CT

MFADD scscFv

Nucleotide sequence:

ggttgatgaccttcgaacatggaccattcctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatctgatggagctca
40 agttcttgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacggtgctgctggagca
gaacgacctggagcgcgggcacaccgggtgctgcgcgagttgctggcctcgtgcgccgacacgatctactgcagcgcctgga
cgacttcgaggcggggacggcgaccgctgcggcccgggggaggcagatctgcaggtggcatttgacattgtgtgacaatgtg
45 gggagagactggaaaagactgccccgcgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccgaagt
ctgagtgagcgggtaaggaggagagctgaaagtctggaagaatgctgagaagaagaacgcctcgggtggccggactggtcaaggcg

ctgcggacctgcaggctgaatctggaggctgacctggagggaagcccaggaatctgtgagcaagagtgagaatatgtccccagt
actaagggttcaactgtgtcttctcagaacacctgactcagatcgat

Amino acid sequence:

5 Vvdpsnmdpflvllhslsgslsgndlmelkflcrervskrklerqvsgldlftvlleqndlerghtgllrellaslrhdllqrllddfea
gtataappgeadlqvafdivcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglikalrtc
rlnlvadlveeaqesvksenmspvldstvsstetp

10 114. 2e12 scFV (SSS-P)H P238S CH2 WCH3-mFADD-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaaggtgtgaatattatgtcacaagtta
atgcagtggtagcaacagaaaccaggacagccacccaaactcctcatctgtgcatccaacgtagaatctggggctccctgccagg
15 tttagtggcagtgggtctgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
aggaaggttcttggacgttcggtggagggcaccaagctggaaatcaaacggggtggcgggtggctcgggaggaggtgggtcgggt
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctgggtggcggcctcacagagcctgtccatcacatgcacctgtc
cagggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtgggtgggaatgatatgggggtg
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaaatgaa
20 cagtctgcaaactgatgacacagccagataactgtgccagagatggtatagtaactttcattactatgttatggactactgggtgcaa
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
tgggtggatcgtcagttcttcttcccccaaaacccaaggacacctcatgatctcccggaccttgaggtcacatgcgtgggtgggt
ggacgtgagccacgaagacctgagggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
ggaggagcagtagaacagcacgtacctgtggtcagcgtcctaccgtctgcaccaggactgggtgaatggcaaggagtacaag
25 tgcaagggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt
acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcc
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcctgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacatggaccattcctggtgctgctgcactcgtgt
30 ccggcagcctgtcgggcaacgatctgatggagctcaagttcttgtccgcgagcgcgtgagcaaacgaaagctggagcgcgtgca
gagtggcctggacctgttcacgggtgctgctggagcagaacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggc
ctcgtgctgcggcagacgatctactgcagcgcctggacgacttcgagggcggggacggcgaccgctgcggcccgggggaggcag
atctgcaggtggcatttgacattgtgtgtgacaatgtggggagagactggaaaagactggccgcgagctgaaggtgtctgaggcc
aagatggatgggattgaggagaagtacccccgaagtctgagtgcgggtaaggagagctgaaagtctggaagaatgctgaga
35 agaagaacgcctcggtggcggactggtcaaggcgtgcggacctgcaggctgaatctgggtggctgacctggtggaagaagccc
aggaatctgtgagcaagagtgagaatatgtccccagtactaagggttcaactgtgtcttctcagaacacctgactcagatcgat

Amino acid sequence:

mdfqvqifsfllisasvimsvrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
40 arfsgsgsgtdfslnihpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit
ctvsgfsltgygvnwvrpppgkglewlgmiwgdgstdynsalksrsltkdsksqvflkmnslqtdtdaryycardgysnfh
yyvmdywgqgtsvtvssdlepkssdkthtspspapellggssvflfppkpkdtlmisrtpetvcvvvdvshedpevkfnwy
vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgqprepvytlppsrdeltn
qvsltclvkgyfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp
45 gkadpsnmdpflvllhslsgslsgndlmelkflcrervskrklerqvsgldlftvlleqndlerghtgllrellaslrhdllqrllddfea

gtataappgeadlqvafdivcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglikalrtc
rlnlvadlveeaquesvksenmspvldstvsstetp

115. 2e12 scFv (SSS-P)H WCH2WCH3-mFADD-TM/CT

5 Nucleotide sequence:

aagcttatggatttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
atctccagcttcttggctgtgtcttaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaagtta
atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgccagg
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
10 aggaaggttcttggacgttcggtggagggaccaagctggaaatcaaacggggtggcgtggctcgggcggaggtgggtcgggt
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctgggtggcgccctcacagagcctgtccatcacatgcacctgt
cagggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
atggaagcacagactataattcagctctcaaatccagactgagcatccaaggacaactccaagagccaagtttcttaaaaatgaa
cagctctgcaaactgatgacacagccagataactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa
15 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
tgggtggaccgtcagttctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtggt
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
ggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag
tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctcgaagccaaagggcagccccgagaaccacaggtgt
20 acacctgccccatccgggatgagctgaccaagaaccagggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc
gccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcttcttcc
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctcctgtctccgggtaaaagcggtaccttcgaacatggaccattcctgtgtgctgctgcactcgtgt
ccggcagcctgtcgggaacgatctgatggagctcaagttctgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgca
25 gagtggcctggacctgttcacggtgctgctggagcagaacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggc
ctcgtcgcgccacacgatctactgcagcgcctggacgacttcgaggcggggacggcgaccgctgcgccccgggggaggcag
atctgcaggtggcatttgacattgtgtgtgacaatgtggggagagactggaaaagactggccgcgagctgaaggtgtctgaggcc
aagatggatgggattgaggagaagtacccccgaagtctgagtgcgggtaaggagagtgctgaaagtctggaagaatgctgaga
agaagaacgctcggtggcggactggtcaaggcgtgcggacctgcaggctgaatctggtggctgacctggtggaagaagccc
30 aggaatctgtgagcaagagtgaataatgtccccagtactaagggttcaactgtgtcttctcagaaacacctgactcagatcgat

Amino acid sequence:

mdfqvqifsfllisasvimrsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
arfgsgsgtdfslnihpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgqvlkesgpglvapsqslsit
35 ctvsgfsltgygvnwvrpppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqddtaryycardgysnfh
yyvmdywggqtsvtvssdlepkssdkhtspspapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwy
vdgvevhnaatkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrdeitkn
qvsltclvkgfypsdiavewesngqpennyktppvldsdgsflyskltvdksrwqqgnvfscsvmhcalhnhytqkslslsp
gkadpsnmddpflvllhslsgslsgndlmelkflcrervskrklervqsgldlftvlleqndlerghtgllrellaslrhdllqrlddfea
40 gtataappgeadlqvafdivcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglikalrtc
rlnlvadlveeaquesvksenmspvldstvsstetp

116. mcasp3-TM/CT

Nucleotide sequence:

45 Ggatccttcgaacatggagaacaacaaaacctcagtgattcaaaatccattaataatttgaagtaaagaccatacatgggagcaag
tcagtggaactctgggatctatctggacagtagttacaaaatggattatcctgaaatgggcatatgcataataataataagaacttcc

ataagagcactggaatgtcatctcgctctggtagcgatgtggacgcagccaacctcagagagacattcatgggcctgaaataccaag
tcagggaataaaaatgatcttactcgtgaagacattttggaattaatggatagtggttctaagggaagatcatagcaaaaggagcagcttgt
gtgtgtgattctaagccatggtgatgaaggggtcatttatgggacaaatgggcctgtgaactgaaaaagtgactagcttcttcagag
gcgactactgccggagtgctgactggaaagccgaaactcttcattcaggcctgccggggtacggagctggactgtggcattgaga
5 cagacagtgggactgatgaggagatggcttgcagaagataccggtggagggtgacttcctgtatgcttactctacagcacctggta
ctattcctggagaaattcaaaggacgggtcgtggttcacccagtcctttgcagcatgctgaagctgtacgcgcacaagctagaatttat
gcacattctcactcgcgttaacaggaaggtggcaacggaattcagtccttctccctggactccactttccacgcaaaagaaacagatc
ccgtgtattgttccatgctcacgaaagaactgtactttatcactagctcgagatcgatg

- 10 Amino acid sequence:
dpsnmennktsvdsksinnfevktihsksvdsgiyldssykmdypemgiciiiiinnknfhkstgmssrsgtdvdaanlretfm
glkyqvrnkndltredilelmdsvskedhskrssfvclshgdegviyngpvelkktsffrgdyrsltgkpklfiiqacrgte
ldcietdsdtdeemacqkipveadflyaystapgyyswrnskdgswfiqslcsmklyahklefmihltrvnrkvatefesfsl
dstfhakkqipcivsmllkelyfyh

15
117. 2e12 scFv (SSS-P)H WCH2WCH3-mcasp3-TM/CT
Nucleotide sequence:

- aagcttatggattttcaagtgcagattttcagcttctgtaatcagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca
atctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaggtgtgaatattatgcacaagtta
20 atgcagtggtagcaacagaaaccaggacagccaccaactcctcctatctctgctcatccaacgtagaatctggggtccctgccagg
tttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaagt
aggaaggttccttggacgttcgggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcggcgagggtgggtcgggt
ggcgcgcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
25 atggaagcacagactataattcagctctcaaatccagactgagcatccaaggaactccaagagccaagttttctaaaaatgaa
cagctctgcaaaactgatgacacagccagataactactgtgccagagatgggtatagtaactttcattactatgttatggactactgggtcaa
ggaaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcc
tgggtggaccgtcagttcttcttccccccaaaacccaaggacacctcatgatctcccggaccttgaggtcacatgcgtggtggt
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
30 ggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctaccgtctgcaccaggactggctgaatggcaaggagtacaag
tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatccaaagccaaagggcagccccgagaaccacaggtgt
acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggctcaaaggcttctatcccagcgacatc
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcc
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtttctcatgtccgtgatgcatgaggctctgcacaac
35 cactacacgcagaagagcctctccctgtctccgggtaaagcgatccttcgaacatggagaacaacaaacctcagtggaatcaaaa
tccattaataattttgaagtaaaagaccatacatgggagcaagtcagtggtactctgggatctatctggacagtagttacaaaatggattatc
ctgaaatgggcatatgcataataattaataaagaacttcataagagcactggaatgtcatctcgtcttggtacggatgtggacgca
gccaacctcagagagacattcatgggcctgaaataccaagtcaaggaataaaaatgatcttactcgtgaagacattttggaattaatgga
tagtggttctaaggaagatcatagcaaaaggagcagcttgtgtgtgtgattctaagccatggtgatgaaggggtcatttatgggacaaa
40 tgggcctgttgaactgaaaaagttgactagcttctcagaggcgactactgccggagtctgactggaaagccgaaactcttcattc
aggcctgccggggtacggagctggactgtggcattgagacagacagtgggactgatgaggagatggcttgcagaagataccgggt
ggaggctgacttctgtatgcttactctacagcacctggttactattcttgagaaattcaaaggacgggtcgtggttcacccagtcctt
tgcagcatgctgaagctgtacgcgcacaagctagaatttatgcacattctcactcgcgttaacaggaaggtggcaacggaattcagat
ccttctccctggactccactttccacgcaaaagaaacagatcccgtgtattgtgtccatgctcacgaaagaactgtactttatcactagct
45 cgagatcgatg

Amino acid sequence:

mdfqvqifsflisasvimsgvdivltqspaslavslgqratiscrasesveyvtslmqwyqqkpgqppkllisaasnvesgvp
arfsqsgsgtdfslnihpveeddiamyfcqqrkvpwtfgggtkleikrgggsgggsgggsgqvlkesgpglvapsqslsit
ctvsgfsltygvnwvrqppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqtdtdaryycardgysnfh
5 yyvmdywgqgtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwy
vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvylppsrldeltn
qvsltlvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslslsp
gkadpsnmennktsvdsksinnfevktihgsksvdsgiyldssykmdypemgiciiiinnknfhkstgmssrsgtdvdaanlre
tfmgikyqvmkndltredilelmdsvskedhskrssfvcvilshgdegviygtngpvelkkltffrgdycrsltgkpklfiiqacr
10 gteldcgietdsgtdeemacqkipveadflyaystapgyyswrmskdgswfiqslcsmklyahklefmhiltrvnrkvatefes
fslstdfhakkqipcivsmiltkelyfyh

118. 2e12 scFv (SSS-P)H P238SCH2WCH3-mcasp3-TM/CT

15 Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaaagtgtgaatattatgcacaagtta
atgcagtggtaccaacagaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggtccctgccagg
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaat
20 aggaaggttccttgacgttcgtggagggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggt
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctggcggcctcacagagcctgtccatcacatgcaccgtct
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggtgggaatgatatggggtg
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtgttcttaaaatgaa
cagctctgcaaatgatgacacagccagatactactgtgccagagatggtatagtaacttcttactatgttatggactactggggtcaa
25 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
tgggtggatcgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgctgtgtggt
ggacgtgagccacgaagacctgaggtcaagttcaactgttacgtggacggcgtggaggtgcataatgcaagacaaagccgcg
ggaggagcagtaaacagcacgtaccgtgtgtgcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag
tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt
30 acacctgcccccatcccggtgatgagctgaccaagaaccagggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc
gccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtggactccgacggctccttctcc
tctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctcctgtctccgggtaaagcgatccttgaattcgaacatggagaacaacaaacctcagtggtat
tcaaatccattaataattttgaagtaaagaccatacatgggagcaagtcagtgactctgggatctatctggacagtagttacaaatg
35 gattatcctgaaatgggcatatgcataataattaataaagaactccataagagcactggaatgtcatctcgtctgtgtacggatgtgg
acgcagccaacctcagagagacattcatgggcctgaaataccaagtcaggaataaaaatgatcttactcgtgaagacattttggaatta
atggatagtgttctaaggaagatcatagcaaaaggagcagctttgtgtgtgattctaagccatggtgatgaaggggtcatttatggg
acaaatgggcctgttgaaactgaaaaagttgactagcttcttcagaggcgactactgccggagtctgactggaaagccgaaactctca
tcattcaggcctgccgggttacggagctggactgtggcattgagacagacagtgaggactgatgaggagatggcttgccagaagata
40 ccggtggagggtgacttctgtatgcttactctacagcacctggttactattcctggagaaattcaaaaggacgggtcgtggtcatccag
tccctttgcagcatgctgaagctgtacgcgcacaagctagaatttatgcacattctactcgcgttaacaggaaggtggcaacggaatt
cgagtccttctccttgactccacttccacgaaagaacagatcccgtgtattgtgtccatgctcacgaaagaactgtactttatcac
tagctcgagatcgatga

45 Amino acid sequence:

mdfqvqifsflisasvimsgvdivltqspaslavlsgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
 arfsgsgsgtdfslnihpveeddiamyfcqqsrvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit
 ctvsgfsltygvnwvrpppgklewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqtddtaryycardgysnfh
 yyvmdywggqtsvtvssdlepkssdkthtspspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy
 5 vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltkn
 qvsltlvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp
 gkadpsnsnmennktsvdsksinnfevktihgksvdsgiyldssykmdypemgiciniinnknfhkstgmssrgtdvdaan
 lretfmglkyqvrnkndltredilelmdsvskedhskrssfvcvilshgdegviygtngpvelkklsffrgdycrsltgkpklfiiq
 acrgtelcgiestdsgtdeemacqkipveadflyaystapgyyswrnskdgswfiqslcsmklyahklefmlhiltrvrnkivate
 10 fesfslstdstfhakkqipcivsmlltkelyfyh

119. mcasp8-TM/CT

Nucleotide sequence:

ttcgaacatggatttccagagttgtctttatgctattgctgaagaactgggcagtgagacctggctgccctcaagttcctgtgcttgac
 15 tacatcccacacaagaagcaggagaccatcgaggatgccagaagctatttctgaggctgcgggaaaaggggatgttgagggaag
 gcaatctgtcttctgaaagagctgctttccacatcagtcggtgggacctgctggtcaacttctagactgcaaccgagaggagatg
 gtgagagagctgcgggatccagacaatgccagatttctccctacagggatgctctttaaagctctcagaagaagtgcgagttgg
 aattgagatcttttaagttcctttgaacaatgagatcccaaatgtaagctggaagatgacttgagcctgcttgaaatgttagaaatg
 gagaagaggaccatgctggcagaaaataacttgaaaccctaaaatcaatctgtgaccaggtcaacaagagcctgctggggaagat
 20 cgaggattatgaaagatcaagcacagagagaagaatgagcctgaaggaagggaagagtgccaccttcagtttggatgagatga
 gcctcaaatggcggaactgtgtgactgccaaagagaacaagacagtgagtcacggacttcagacaaagtaccaaatgaagaac
 aaacctcggggatactgtctgatcatcaacaatcatgatttcagcaaggccgggaagacataaccaactccgaaaaatgaaggac
 agaaaaggaaacagactgtgataaagaggctctgagtaagaccttaaggagcttcattttagatagtagtcttacgacgactgcactgc
 aatgaaatccacgagattctagaaggctaccaaaagcgcagaccacaagaacaagactgcttcactgtctgtatctatcccacggt
 25 gacaagggtgtcgtctatggaacggatgggaaggaggcctccatctatgacctgacatcttacttactggttcaaatgcccttcct
 gtctgggaaaccaagatcttttattcaggcttgccaaggaagtaacttcagaaaggagtgctgatgaggcaggcttcagcaa
 cagaaccacactttagaagtggattcatctctcacaagaactatattccggatgaggcagactttctgctgggaatggctacggtgaa
 gaactgcgttctaccgagatcctgtgaatggaacctggtatattcagtcactttgccagagcctgagggaagatgtctcaaggag
 atgacattcttagcatcctgactggcgtgaactatgacgtgagcaataaagacgacaggaggaacaagggaagcagatgccacag
 30 cccaccttcacactacggaagaagctcttctccctccctaagctcagatcgatt

Amino acid sequence:

snmdfqsclyiaaeelgsedlaalkflclidyiphkkqetiedaqklflrlrekgmleegnlsflkellfhisrwdllvnfldcnreem
 35 vrelrdpdnaqispyrvmlflklseevselelrsfkflinneipckckleddlsleifvemekrtmlaennletlksicdqvnskllgki
 edyerssterrmslegreelpsvldemslkmaelcdspreqdsesrtsdkvyqmknkprgycliinnhdfskareditqlrk
 kdrkgtdcdkealsktfkelhfeivsyddctaneiheilegyqsadhknkdcficcilshgdkgvvygtdgkeasiydltsyftgs
 kcpslsgkpkiffiqacqgsnfqkgvpdeagfeqqnhtlevdssshknyipdeadfllgmatvkncvsyrdpvnngtwyiqslc
 40 qslrercpqgddilsiltgvnydvsnkddrnkgkqmpqptflrklffpp

120. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp8-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacca
 45 atctccagcttctttggctgtgtctctagtcagagagccaccatctcctgcagagccagtgaaagtgttgatattatgcacaagtta
 atgcagtgttaccacagaaaccaggacagccaccaaacctcctcatctgtgcatccaacgtagaatctggggtccctgcagg

tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
 aggaagggtccttgacgttcggtggaggcaccaagctggaaatcaaacgggggtggcgggtggctcgggagggtgggtcgggt
 ggcggggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
 5 atggaagcacagactataattcagctctcaatccagactgagcatcaccaaggacaactccaagagccaagtttctaaaaatgaa
 cagctctgaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtgcaa
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
 tgggtggaccgtcagttctcttccccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtgggtggt
 ggacgtgagccacgaagaccctgaggtaagttcaactggtacgtggacggcgtggagggtgcataatgccaaagacaaagccgcg
 10 ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaag
 tgcaaggtctcaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgt
 acacctgccccatcccgggatgagctgaccaagaaccagggtcagcctgacctgcctggtcaaggcttctatcccagcgacatc
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcc
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgcatgaggctctgcacaac
 15 cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacatggatttcagagttgtctttatgctattgctgaa
 gaactgggcagtgaaagacctggctgccctcaagttcctgtgcttggactacatcccacacaagaagcaggagaccatcgaggatgc
 ccagaagctatttctgaggctgcgggaaaagggtggtggaggaaaggcaatctgtcttctgaaagagctgctttccacatcagtc
 ggtgggacctgctggtcaacttccttagactgcaaccgagaggagatggtgagagagctgcgggatccagacaatcccagatttct
 ccctacagggtcatgctcttaagctctcagaagaagtgagcgagttggaattgagatctttaagttcctttgaacaatgagatcccca
 20 aatgtaagctggaagatgacttgagcctgcttgaaattttgtagaatggagaagaggaccatgctggcagaaaataacttggaac
 ctaaaatcaatctgtgaccaggtcaacaagagcctgctggggaagatcgaggattatgaaagatcaagcacagagagaagaatga
 gccttgaaggaagggaagagttgccacctcagtttggatgagatgagcctcaaatggcggaactgtgtgactcgccaagagaac
 aagacagtgagtcacggacttcagacaaagtttaccaaatgaagaacaaacctcggggatactgtctgatcatcaacaatcatgattc
 agcaaggcccggaagacataaccaactccgaaaaatgaaggacagaaaagggaacagactgtgataaaggctctgagtaag
 25 accttaaggagcttattttgagatagtatcttacgacgactgcactgcaaatgaaatccacgagattctagaaggctaccaaaagcgc
 agaccacaagaacaaagactgcttcatctgctgtatcctatcccacgggtgacaagggtgtcgtctatggaacggatgggaaggaggc
 ctccatctatgacctgacatcttacttactggttcaaagtgccttccctgtctgggaaacccaagatcttttattcaggcttgccaag
 gaagtaacttcagaaaggagtgctgatgaggcaggttcgagcaacagaaccacacttagaagtgattcatctcacaaga
 actatattccggatgaggcagactttctgctgggaatggctacgggtgaagaactgcgttctaccgagatcctgtgaatggaacctgg
 30 tatattcagtcactttgccagagcctgagggaaagatgtcctcaaggagatgacattcttagcatcctgactggcgtgaactatgacgt
 gagcaataaagacgacaggaggaacaagggaagcagatgccacagcccaccttcacactacggaagaagctcttctccctccc
 taatgactcagatcgatt

Amino acid sequence:

35 mdfqvqifsfllisasvimsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
 arfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit
 ctvsgfsltgygvnwvrppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqddtaryycardgysnfh
 yyvmdywggtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmisrtpevtcvvdvshedpevkfnwy
 vdgvevhnaaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltn
 40 qvsltlvkgfypsdiavewesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp
 gkadpsnmfdqsclyiaaelgsedlaalkflcldyiphkkqetiedaqklflrrekgmleegnlsfkellfhisrwdllvnfldc
 nreemvrelrdpnaqispyrvmlfklseevselelrsfkflnneipkckleddlsleifvemekrtmlaennletlksicdqv
 kslgkiedyersstermslegreelppsvldemslkmaelcdspreqdsesrtsdkvyqmknkprgycliinnhdfskaredi
 tqlrkmkdrkgtdcdkealsktfkelhfeivsyddctaneiheilegyqsadhknkdcficilshgdkgvvygtdgkeasiydl
 45 syftgskcpslsgkpkiffiqacqgsnfqkgvpdeagfeqqnhtlevdssshknyipdeadflgmatvkncvsyrdpvngrw
 yiqslcqlsrercpqgddilsiltgvnydvsnkddrrnkkgkmpqptflrkklffpp

121. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-mcasp8-TM/CT

Nucleotide sequence:

5 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca
atctccagcttctttggctgtgtctctaggtcagagagccaccatctctcgagagccagtgaaagtgtgaatattatgtcacaagttta
atgcagtggtagcaacagaaaccaggacagccaccaaaactcctcatctctgctgcatccaacgtagaatctgggggtccctgccagg
tttagtggcagtggtgtgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
aggaaggttccttgacgttcggtggaggccaccaagctggaaatcaaacggggtggcgggtggtcgggaggagtggtgggtgggt
ggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgcctcacagagcctgtccatcacatgcaccgtct
10 cagggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
atggaagcacagactataattcagctctcaaatccagactgagcatccaaggaactccaagagccaagtttttaaaaatgaa
cagctctgcaaactgatgacacagccagataactactgtgccagagatgggtatagtaactttcattactatgttatggactactggggtcaa
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacatcccaccgtcccagcacctgaactcc
tgggtggatcgtcagttcttcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggt
15 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggagggtgcataatgccaagacaaagccgcg
ggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtagaag
tgcaaggtctccaacaaagccctccagcccccatcgagaaaaccatccaaagccaagggcagccccgagaaccacaggtgt
acacctgcccccatccgggatgagctgaccaagaaccagggtcagcctgacctgcctggtcaaaggcttctatccagcgacatc
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctggtgactccgacggctccttcttcc
20 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctcctgtctccgggtaaagcgatccttcgaacatggatttcagagttgtctttatgctattgctgaa
gaactgggcagtgaaacctggctgccctcaagttcctgtgcttgactacatcccacacaagaagcaggagaccatcgaggatgc
ccagaagctatttctgaggctgcgggaaaaggggatgttgagggaaggcaatctgtcttctgaaagagctgctttccacatcagtc
gggtgggacctgctggtcaacttcttagactgcaaccgagaggagatggtgagagagctcggggatccagacaatgccagatttct
25 ccctacagggtcatgctcttaagctctcagaagaagtgaagcaggttggaattgagatctttaagttcctttgaaatgagatcccca
aatgtaagctggaagatgacttgacctgcttgaaattttgtagaatggagaagaggaccatgctggcagaaaataacttggaac
cctaaaatcaatctgtgaccaggtcaacaagacgtctggtgggaagatcgaggattatgaaagatcaagcagagagaagaatga
gccttgaaaggaagggaagattgccacctcagttttgatgagatgagcctcaaaatggcggaactgtgtgactcgccaagagaac
aagacagtgagtcacggacttcagacaaagttaccaaatgaagaacaacctcggggatactgtctgatcatcaacaatcatgatttc
30 agcaaggcccggaagacataacccaactccgaaaaatgaaggacagaaaaggaacagactgtgataaagggtctgagtaag
acctttaaggagcttcatttgagatagtattacgacgactgcactgcaaatgaaatccacgagattctagaaggctaccaaaagcgc
agaccacaagaacaaagactgcttcatctgctgtatcctatcccacggtgacaagggtgtctgtatggaacggatgggaaggaggc
ctccatctatgacctgacatcttacttactggttcaagtgcccttcctgtctgggaacccaagatcttttattcagggttgccaag
gaagtaacttcagaaaggagtgcctgatgaggcaggcttcgagcaacagaaccacactttaagtgattcatcatctcacaaga
35 actatattccggatgaggcagactttctgctgggaatggctacgggtgaagaactcgcttctaccgagatcctgtgaatggaacctgg
tatattcagtcactttgccagagcctgagggaaagatgtcctcaaggagatgacattcttagcatcctgactggcgtgaactatgacgt
gagcaataaagacgacaggaggaacaagggaagcagatgccacagcccaccttcacactacggaagaagcttcttccctccc
taatgactcgagatcgattc

40 Amino acid sequence:

mdfqvqifsfllisasvmsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
arfsrgsgtdfslnihpveeddiamyfcqqrkvpwtfgggtkleikrgggsgggsgggsgqvlkesgpglvapsqslsit
ctvsgfsltygvnwvrppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqddtaryycardgysnfh
yyvmdywgqgtsvtvssdlepkssdkhtsppspapellggssvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwy
45 vdgevehnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeitkn
qvsltlvkgfypsdiavewesngqpennkytppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp

gkadpsnmdfqsclyaiaeelgsedlaalkfclldyiphkktetiedaqklflrlrekgmleegnlsfklkellfhisrwdllvnfldc
 nreemvrelrdpdnaqispyrvmlfklseevselelrsfklflnneipkckleddlsleifvemekrtmlaennletlksicdqv
 kslgkiedyerssterrmslegreelpsvldemslkmaelcdspreqdsesrtsdkvyqmknkprgycliinnhdfskaredi
 tqlrkmkdrkgtdcdkealsktfkelhfeivsyddctaneiheilegyqsadhknkdcficcilshgdkgvvygtgkeasiydl
 5 syftgskcpslsgkpkiffiqacqgsnfqkvpdeagfeqqnhtlevdssshknyipdeadflgmatvkncvsyrpvngrw
 yiqslcqlsrercpqgddilsiltgvnydvsnkddrrnkqkmpqptflrkkllfpp

122. hcas3-TM/CT

10 Nucleotide sequence:

Ggatccttcgaacatggagaacactgaaaactcagtgattcaaaaatccattaaaaattggaaccaagatcatacatggaagcga
 atcaatggactctggaatatccctggacaacagttataaatggattatcctgagatgggtttatgtataataattaataagaatttca
 taaaagcactggaatgacatctcggctcgttacagatgcatgcagcaaacctcagggaacattcagaaacttgaaatatgaagtc
 aggaataaaaaatgatcttacacgtgaagaaattgtggaattgatgcgtgatgtttctaaagaagatcacagcaaaaggagcagtttgtt
 15 tgtgtgcttctgagccatggtgaagaagggaataattttggaacaaatggacctgtgacctgaaaaaataacaaacttttcagagg
 gatcgttgtagaagttaactgaaaacccaaactttcattattcaggcctgccgtgtacagaactggactgtggcattgagacaga
 cagtgggtgatgatgacatggcgtgtcataaaataccagtgaggccgacttctgtatgcatactccacagcacctggtattattct
 tggcgaattcaaaaggatggctcctgggtcatccagtcgctttgtgccatgctgaaacagtatgccgacaagcttgaatttatgcacatt
 cttaccgggttaaccgaaagggtggaacagaatttgagtccttttcttgacgctacttttcatgcaagaacagattccatgtattgt
 20 ttccatgctcacaagaactctatttttactactaactcagatcgata

Amino acid sequence:

dpsnmentensvdsksiknlepkiihgsesmdsgisldnsykmdypemglciiinnknfhkstgmtsrsgtdvdaanlretfrn
 lkyevrnkndltreeivlmrdvskedhskrsvfcvllshgeegiiifgtngpvdllkitnffrgdrslrtgkpkllfiiaqrgetld
 25 cgietsdgvdddmachkipveadflyaystapgyyswrnskdgswfiqslcamlkqyadklefmhiltvrnkratefesfsfd
 atfhakkqipcivsmlltelyfyh

123. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcas3-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacca
 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta
 atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgtcgcacccaacgtagaatctggggtccctgccagg
 tttagtggcagtggtctgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtatttctgcagcaaat
 aggaaggttcttgacgttcggtggagggaccaagctggaatcaaacggggtggcgggtggctcgggaggaggtgggtcgggt
 35 ggcgggcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcggcctcacagagcctgtccatcacatgcaccgtct
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
 atggaagcacagactataattcagctctcaaatccagactgagcatccaagacaactccaagagccaagtttcttaaaaatgaa
 cagctctgaaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactggggtcaa
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
 40 tgggtggaccgtcagcttctcttccccccaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtggtggt
 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaagacaaagccgcg
 ggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaag
 tgcaaggctccaacaaagccctccagcccccatcgagaaaaccatctcaaaagccaaagggcagccccgagaaccacaggtgt
 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgcaaaaggcttctatcccagcgacatc
 45 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcttcttcc
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac

cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacatggagaacactgaaaactcagtggaattcaaaa
 tccattaaaaatttggaaacaaagatcatacatggaagcgaatcaatggactctggaatatccctggacaacagttataaaatggattat
 cctgagatgggtttatgtataataattaataagaattttcataaaagcactggaatgacatctcggtctggtacagatgtcgtatgcag
 caaacctcagggaaacattcagaaacttgaaatatgaagtcaggaataaaaatgatcttacacgtgaagaaatttggaattgatgcgt
 5 gatgtttctaaagaagatcacagcaaaaggagcagttttgttgtgtgcttctgagccatggtgaagaaggaataattttggaaacaaat
 ggacctgttgacctgaaaaaataacaaactttttcagaggggagcgtttagaagcttaactggaaaacccaaacttttcattattcag
 gcctgccgtggtacagaactggactgtggcattgagacagacagtggtgttgatgacatggcgtgtcataaaataccagtggag
 gccgacttctgtatgcatactccacagcacctgggtattattcttggcgaaattcaaaggatggctcctggttcaccagtcgcttgtgc
 catgctgaaacagtatgccgacaagcttgaatttatgcacattcttaccgggtaaccgaaaggtggcaacagaatttgagtccttttc
 10 ctttgacgctacttttcagcaagaacagattccatgtattgtttccatgctcacaaaagaactctattttatcactaactcgagatcgat
 a

Amino acid sequence:

mdfqvqifsflisasvmsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
 15 arfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit
 ctvsgfsltgygvnwvrppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqtdtdaryycardgysnfh
 yyvmdywggqtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy
 vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgpprepqvytlppsrdeltkn
 qvsltlvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp
 20 gkadpsnmentensvdsksiknlepkiihgsesmdsgisldnsykmdypemglciiinnknfhkstgmtsrsgtdvdaanle
 tfmllkyevmknldtreeivelmrdvskedhskrssfvcvllshgeegii fgtngpvdllkitnffrgdrsrsltgkpklfiiqacrgt
 eldcgietsgvdmdmachkipveadflyaystapgyyswmksdgswwfiqslcamlkqyadklefmhiltrvnrkvatefesf
 sfdatfhakkqipcivsmlltkelyfyh

25 123. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp3-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
 atctccagcttctttggctgtgtctctaggtcagagagccaccatctctcgagagccagtgaaagtgtgaatattatgcacaagttta
 atgcagtggtaccaacagaaaccaggacagccaccaaacctcctcatctctgctgcatacaacgtagaatctggggtccctgccagg
 30 tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaat
 aggaaggttcttgacgttcggtggagggaccaagctggaaatcaaacggggtggcgtggctcggcgagggtgggtcgggt
 ggcgggcgagatcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct
 caggggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctaaaaatgaa
 35 cagctctgcaaactgatgacacagccagatactactgtgccagagatgggtatagtaactttcattactatgttatggactactggggtcaa
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
 tgggtggatcgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtggt
 ggacgtgagccacgaagaccctgaggtcaagttcaactgggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
 ggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactgggtgaatggcaaggagtacaag
 40 tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaagggcagccccgagaaccacaggtgt
 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaagggttctatcccagcgacatc
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcc
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgaggctctgcacaac
 cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacatggagaacactgaaaactcagtggaattcaaaa
 45 tccattaaaaatttggaaacaaagatcatacatggaagcgaatcaatggactctggaatatccctggacaacagttataaaatggattat
 cctgagatgggtttatgtataataattaataagaattttcataaaagcactggaatgacatctcggtctggtacagatgtcgtatgcag

caaacctcagggaaacattcagaaacttgaaatatgaagtcaggaataaaaatgatcttacacgtgaagaaattgtggaattgatgcgt
 gatgtttctaagaagatcacagcaaaaggagcagttttgtgtgcttctgagccatggtgaagaaggaataatgttgaacaaat
 ggacctgttgacctgaaaaaataacaaacttttcagaggggagcgtttagaagtcacttgaaaacccaaacttttcattattcag
 gcctgccgtggtacagaactggactgtggcattgagacagacagtggtgtgatgatgacatggcgtgtcataaaataaccagtggag
 5 gccgacttctgtatgcatactccacagcacctggttattattcttggcgaaattcaaaaggatggctcctggttcattcagtcgcttgtgc
 catgctgaaacagtatgccgacaagctgaatttatgcacattcttaccgggtaaccgaaagggtggcaacagaatttgagtcctttc
 ctttgacgctacttttcatgcaaagaaacagattccatgtattgttccatgctcacaaaagaactctattttatcactaactcgagatcgat
 aa

10 Amino acid sequence:

mdfqvqifsflisasvimsvrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
 arfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggkgleikrgggsgggsgggsgvqlkesgpglvapsqslsit
 ctvsgfsltgygvnwvrpppgkglewlgmiwgdgstdynsalksrlsitkdnsksqvflkmnslqddtaryycardgysnfh
 yyvmdywgqgtsvtvssdlepkssdkthtspspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy
 15 vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgpprepqvylppsrdeltn
 qvsltlvkgfypsdiavewesngqpennyktpvpldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp
 gkadpsnmentensvdsksiknlepkiihgsesmdsgisldnsykmndypemglciiinnknfhkstgmtsrsgtdvdaanre
 tfmlkyevrnkndltreeivelmrdivskedhskrssfcvllshgeegiifgtngpvdllkitnffrgdrsrsltkgpklfiiqacrgt
 eldcgietsdgvdddmachkipveadflyaystapgyyswmnskdgswfiqslcamlkqyadklefmhiltrvnrkvatefesf
 20 sfdatfhakkqipci vsmltkelyfyh

124. hcasp8-TM/CT

hCaspase8B:

Nucleotide sequence:

25 ggatccttcgaacatggacttcagcagaaactttatgatattggggaacaactggacagtgaagatctggcctccctcaagttcctga
 gcctggactacattccgaaaggaagcaagaacccatcaaggatgccttgatgtattccagagactccaggaaaagagaatgttg
 aggaaagcaatctgtccttctgaaggagctgctctccgaattaatagactggatttctgattacctacctaactagaaaggag
 gagatggaaagggaacttcagacaccaggcagggtcctcaaatctgcctacagggtcatgctctatcagatttcagaagaagtgagc
 agatcagaattgaggtctttaagtcttttgaagaggaaatctccaaatgcaaactggatgatgacatgaacctgctggatatttcat
 30 agagatggagaagagggtcatcctgggagaaggaagtggacatctgaaaagagctgtgccaaatcaacaagagcctgctg
 aagataatcaacgactatgaagaattcagcaagagagaagcagcagcctgaaggaagtcctgatgaatttcaaatggggagga
 gttgtgtgggtaatgacaatctcgactctcaagagaacaggatagtgaatcacagactttggacaaagttaacaaatgaaaagc
 aaacctcggggatactgtctgatcatcaacaatcacaatttgcaaaagcacgggagaagtgccaaacttcacagcattaggagac
 aggaatggaacacacttgatgcaggggcttgaccacgaccttgagagcttcatttgagatcaagccccacgatgactgcacag
 35 tagagcaaatctatgagatttgaaaatctaccaactcatggaccacagtaacatggactgcttcatctgctgtatcctctccatggaga
 caagggcacatctatggcactgatggacaggaggccccatctatgagctgacatctcagttcactggttgaagtgccttcccttg
 ctggaacccaaagtgtttttattcaggctgtcagggggataactaccagaaaggtatacctgttgagactgattcagaggagcaa
 ccctatttagaaatggatttatcatcacctcaaacgagatatatccggatgaggctgactttctgctggggatggccactgtgaataact
 gtgttccctaccgaaaccctgcagaggggaacctgggtacatccagtcactttgccagagcctgagagagc gatgtcctcgaggcgatg
 40 atatttcaccatcctgactgaagtgaactatgaagtaagcaacaaggatgacaagaaaaacatggggaaacagatgcctcagccta
 ctttcacactaagaaaaaaactgtcttccctctgattgagcatgcatcgata

Amino acid sequence:

Dpsnmdfsmlydigeqlsdslaslkflsldyipqrkqepikdalmlfqlrlekrmlaesnlslkellfrinrldllitylntrkee
 45 merelqtpgraqisayrvmlyqiseevsrslrsfkllqeeiskckldddmlldifiemekrvilgegkldilkrvcaqinkslk
 iindyeefskrssslegspdefsngeelcgvmtisdspreqdsesqldkvyqmkskprgycyliinnhnfakarekvpklhsir

drngthldagalttfeelhfeikphddctveqiyeilkiyqlmdhsnmndcficilshgdkgiiygtgdqeapiyeltsqftglkcp
 slagkpkvffiqacqgdnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnvcsyrnpaegtwyiqslcqlr
 ercprgddiltitevnyevsnkddkknmgkqmpqptflrkklvfspd

5 hCaspase8C:

Nucleotide sequence:

ggatccttcgaacatggacttcagcagaaatctttatgatattggggaacaactggacagtgaagatctggcctccctcaagttcctga
 gcctggactacattccgcaaaggaagcaagaacctcataggatgccttgatgttattccagagactccaggaaaagagaatgttgg
 aggaaagcaatctgtccttctgaaggagctgctctccgaattaatagactggatttgctgattacctacctaactagaaaggag
 10 gagatggaaaggggaacttcagacaccaggcagggtcctcaatttctgcctacagggtcatgctctatcagatttcagaagaagtgagc
 agatcagaattgaggtcttttaagtttctttgcaagaggaaatctccaaatgcaaactggatgatgacatgaacctgctggatatttcat
 agagatggagaagagggtcatcctgggagaaggaaagtggacatcctgaaaagagtctgtgcccaaatcaacaagagcctgctg
 aagataatcaacgactatgaagaattcagcaaaggggaggagttgtgtgggtaatacacaatctcggactctccaagagaacaggat
 agtgaatcacagactttggacaaagttaccaaatgaaaagcaaactcggggatactgtctgatcatcaacaatcacaattttgcaa
 15 agcacgggagaaagtgcccaacttcacagcattagggacaggaatggaacacactggatgcaggggctttgaccacgacctttg
 aagagcttcattttgatcaagccccacgatgactgcacagtagagcaaatctatgagatttgaaaatctaccaactcatggaccac
 agtaacatggactgcttcatctgtgtatcctctccatggagacaagggcatcatatggcactgatggacaggaggccccatcta
 tgagctgacatctcagttcactggtttgaagtgcccttccctgctggaaaacccaaagtgtttttattcagcctgtcagggggataact
 accagaaaggtatacctgttgagactgattcagaggagcaacctatttagaaatggatttatcatcacctcaaacgagatatatccc
 20 gatgaggtgactttctgctgggatggccactgtgaataactgtgttctaccgaaacctgcagagggaacctggtacatccagt
 cactttgccagagcctgagagagcgatgtcctcaggcgatgatattctcaccatcctgactgaagtgaactatgaagtaagcaacaa
 ggatgacaagaaaaacatggggaaacagatgcctcagcctactttcacactaagaaaaaacttgcttccctctgattgagcatgca
 tcgata

25 Amino acid sequence:

dpsnmndfsmlydigeqlsdslaslkflsldyipqrkqepikdalmlfqlrleqrmleesnlslfkellfrinrldlitylntrkeem
 erelqtpgraqisayrvmllyqiseevsrslrsfkflqeeiskcklddmnlldifiemekrvilgegkldilkrvcaqinkslkii
 ndyeefskgeelcgvmtisdspreqdsesqtdkvyqmkskprgycliinnhnfakarekvpklhsirdngthldagalttfee
 lhfeikphddctveqiyeilkiyqlmdhsnmndcficilshgdkgiiygtgdqeapiyeltsqftglkcp
 30 dnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnvcsyrnpaegtwyiqslcqlrercprgddiltitevny
 evsnkddkknmgkqmpqptflrkklvfspd

125. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcasp8-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
 35 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaggtgtgaatattatgcacaagttta
 atgcagtggtagcaacagaaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggtccctgccagg
 tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
 aggaaggttcttgacgttcggtggagggcaccaagctggaaatcaaacggggtggcggtggctcgggagggtgggtcgggt
 40 ggcggcggtatcaggtgcagctgaaggagtcaggacctggcctgggtggcgccctcacagagcctgtccatcacatgcaccgtct
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
 atggaagcacagactataattcagctctcaaatccagactgagcatccaaggacaactccaagagccaagttttctaaaaatgaa
 cagtctgcaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactggggtcaa
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcc
 45 tgggtggaccgtcagttctcttccccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtgtggt
 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaagccgcg

ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaag
 tgcaagggtctccaacaaagccctcccagccccatcgagaaaacctctcaaagccaaagggcagccccgagaaccacaggtgt
 acacctgccccatcccgggatgagctgaccaagaaccagggtcagcctgacctgctggtcaaaggcttctatcccagcgacatc
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc
 5 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
 cactacacgcagaagagcctctccctgtctccgggtaagcggatcctcgaacatggacttcagcagaaatcttatgatattgggga
 acaactggacagtgaagatctggcctccctcaagttcctgagcctggactacattccgcaaaggaagcaagaacctatcaaggatg
 ccttgatgttattccagagactccaggaaaagagaatgttggaggaaagcaatctgtccttctgaaggagctgcttctccgaattaata
 gactggatttgctgattacctacctaactagaaaggaggagatggaaaggggaacttcagacaccaggcagggtcctaaattctg
 10 cctacagggtcatgctctatcagatttcagaagaagtgagcagatcagaattgaggtcttttaagtttctttgcaaggaggaaatctcaa
 atgcaaactggatgatgacatgaacctgctggatatttcatagagatggagaagaggggtcatcctgggagaaggaaagttggacat
 cctgaaaagagtctgtgccccaaatcaacaagcctgctgaagataatcaacgactatgaagaattcagcaaagagagaagcagca
 gccttgaaaggaagtcctgatgaatttcaaatggggaggagtgtgtgggtaatgacaatctcggactctcaagagaacaggatag
 tgaatcacagactttggacaaagttaccaaatgaaaagcaaacctcggggatactgtctgatcatcaacaatcacaattttgaaaag
 15 cacgggagaaagtgcccaaacttcacagcattagggacaggaatggaacacacttgatgcaggggcttggaccacgaccttgaa
 gagcttcattttgatcaagccccacgatgactgcacagtagagcaaatctatgagatttgaaaatctaccaactcatggaccacag
 taacatggactgcttcatctgtgtatcctctccatggagacaagggcatcatctatggcactgatggacaggaggccccatctatg
 agctgacatctcagttcacttggttgaagtgcccttccctgctggaaaacccaaagtgtttttattcaggcttgcagggggataactac
 cagaaaaggatatacctgttgagactgattcagaggagcaaccctatttagaaatggatttatcatcacctcaacgagatatatcccgat
 20 gaggtgactttctgctggggatggccactgtgaataactgttttctaccgaaaccctgcagagggaacctggtacatccagtcact
 ttgccagagcctgagagagcgtatgtctcagggcgtatattctaccatcctgactgaagtgaactatgaagtaagcaacaaggat
 gacaagaaaaacatggggaaacagatgcctcagcctacttccactaagaaaaaactgtcttcccttctgattgagcatgcatcga
 ta

25

Amino acid sequence:

mdfqvqifsflisasvimsvrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkillisaasnvesgvp
 arfsgsgsgtdfslnihpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgggsgvqlkesgpglvapsqslsit
 ctvsgfsltgygvnwvrpppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqtdtdaryycardgysnfh
 30 yyvmdywggqtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy
 vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrdeltkn
 qvsltlcvkgfypsdiavewesngqpennyktpvpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsps
 gkadpsnmfdsmlydigeqldsedlaslkflsldyipqrkqepikdalmfqlrleqrmleesnlslfcellfrinrldlitylntrk
 eemerelqtpgraqisayrvmllyqiseevrselrsfkflqeeiskckldddmlldifiemekrvilgegkldilkrvcaqinksl
 35 lkiindyeefskerssslegspdefsngeelcgvmtisdspreqdsesqtdkvyqmkskprgycyliinnhnfakarekvpklhsi
 rdngthldagalttfeelhfeikphddctveiqiyeilkiyqlmdhsnmddficilshgdkgiiygtgdqeapiyeltsqftglkc
 pslagkpkvffiqacqgdnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnncvsyrnpaegtwyiqslcql
 rercprgdiltitevnyevsnkddkknmgkmpqptflrkklvfspd

40

126. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp8B-TM/CT **(other caspase 8 isoforms are similar)**

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacca
 45 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaagtggtgaatattatgcacaagtta
 atgcagtggtaccaacagaacaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggctcctgccagg

tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
 aggaaggttccttgacgttcggtggagggaccaagctggaaatcaaacggggtggcgggtggctcgggagggtgggtcgggt
 ggcgccggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggtgggaatgatatggggtg
 5 atggaagcacagactataattcagctctcaatccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaaatgaa
 cagtctgcaaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtgcaa
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc
 tgggtggatcgtcagcttcttcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggt
 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
 10 ggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag
 tgcaaggtctccaacaaagccctccagcccccatcgagaaaaccatctcaaagccaaagggcagccccgagaaccacaggtgt
 acacctgccccatcccggtgatgagctgaccaagaaccagggtcagcctgacctgcctggtcaaggcttctatcccagcgacatc
 gccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctccgtgctggactccgacggctccttcttcc
 tctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
 15 cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacatggacttcagcagaatcttctatgatattgggga
 acaactggacagtgaagatctggcctccctcaagttcctgagcctggactacattccgcaaagggaagcaagaacctatcaaggatg
 ccttgatgttattccagagactccaggaaaagagaatgttgaggaaagcaatctgtccttctgaaggagctgctctccgaattaata
 gactggatttgctgattacctacctaactagaaaggaggagatggaagggaacttcagacaccaggcagggtcctcaatttctg
 cctacagggtcatgctctatcagatttcagaagaagtgagcagatcagaattgaggtctttaagtttctttgcaaggaggaaatctcaa
 20 atgcaaactggatgatgacatgaacctgctggatatttcatagagatggagaaggaggtcatcctgggagaaggaaagtggacat
 cctgaaaagagctgtgccccaaatcaacaagacgtgctgaagataatcaacgactatgaagaattcagcaaagagagaagcagca
 gccttgaagggaagctgatgaattttcaaatggggaggagtgtgtggggtaatgacaatctcggactctccaagagaacaggatag
 tgaatcacagactttggacaaagtttaccaaatgaaaagcaaacctcggggatactgtctgatcatcaacaatcacaattttgcaaaag
 cacgggagaaagtggccaaacttcacagcattagggacaggaatggaacacacttgatgcaggggctttgaccacgacctttgaa
 25 gagcttcattttgagatcaagccccacgatgactgcacagtagagcaaatctatgagattttgaaaatctaccaactcatggaccacag
 taacatggactgctcatctgctgtatcctctcccatggagacaagggtcatcatatggcactgatggacaggaggcccccatctatg
 agctgacatctcagttcactggttgaagtgccttccctgctggaaaacccaaagtgtttttattcaggcttgcagggggataactac
 cagaaaggatatacctgttgagactgattcagaggagcaaccctatttagaaatggatttatcatcacctcaaacgagatatatccggat
 gaggtgactttctgctggggatggccactgtgaataactgtgttctaccgaaacctgcagagggaacctgtacatccagtcact
 30 ttgccagagcctgagagagcgtatgctcagggcgtgatattctcaccatcctgactgaagtgaactatgaagtaagcaacaaggat
 gacaagaaaaacatggggaacagatgcctcagcctactttcacactaagaaaaaaactgtcttcccttctgattgagcatgcatcga
 taa

35 Amino acid sequence:
 mdfqvqifsfllisasvimrsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyyqqkpgqppklisaasnvesgvp
 arfsgsgsgtdfslnihpveeddiamyfcqqsrvpwtfgggtkleikrgggsgggsgggsgggsgvqlkesgpglvapsqslsit
 ctvsfsltygvnwvrpppgkglewlgmiwgdstdynsalksrslsitkdnsksqvflkmnslqtdtdaryycardgysnfh
 yyvmdywgqgtsvtvssdlepkssdkthtspspapellggssvflfppkpkdtlmsirtpetvvcvvdvshedpevkfnwy
 40 vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlpssrdeltkn
 qvsltlvkgfypsdiavewesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslslsp
 gkadpsnmfdsrnlydigeqlsdslaslkflsldyipqrkqepikdalmlfqlqekrmleesnlslkellfrinrldlitylntrk
 eemerelqtpgraqisayrmlyqiseevsrslrsfkflqeeiskckldddmnlldifiemekrvilgeglkdlkrvcaqinksl
 lkiindyeefskerssslegspdefsngeelcgvmtisdspreqdsesqtdkvyqmkskprgycliinnhnfakarekvpklhsi
 45 rdmngthldagaltttfeelhfeikphddctveqiyelkiyqlmdhsnmddcficilshgdkgiygtgdgeapiyeltsqftglkc

pslagkpkvffiqacqgdnyqkgipvetdseeqpylemldsspqtryipdeadflgmatvnncvsyrnpaegtwyiqslcql
rercprgdiltilevnyevsnkddknmgkmpqptflrklvfpsd

5 **127. hFADD-TM/CT**

Nucleotide sequence:

Gttgtgatccttcgaacccgttctggtgctgctgactcgggtgctgctcagcctgtcgagcagcgagctgaccgagctcaagtcc
tatgcctcgggcgcgtgggcaagcgcaagctggagcgcgtgcagagcggcctagacctcttccatgctgctggagcagaacga
cctggagccccgggcacaccgagctcctgcgcgagctgctgcctccctgcggcgccacgacctgtcgggcgctgcgacgactt
10 cgagggcgggggcgggcgccggggccgcgcctggggaagaagacctgtgtgcagcatttaacgtcatatgtgataatgtggggaa
agattggagaaggctggctcgtcagctcaaagctcagacaccaagatcgacagcatcgaggacagataacccccgaacctgaca
gagcgtgtcgggagtcactgagaatctggaagaacacagagaaggagaacgaacagtgggccacctggtgggggctctcag
gtcctgccagatgaacctggtggctgacctggtacaagaggttcagcaggcccgtagctccagaacaggagtggggccatgtcc
ccgatgtcatggaactcagacgcacatctacctccgaagcgtcctgataactcgagatcgataacaac

15

Amino acid sequence:

Dpsnpflvllhsvssslsssteltelkflclgrvgkrklervqsgldfsmllleqndlepghtellrellaslrhldlrrvddfeagaaag
aapgeedlcaafnvicdnvgkdwrrlarqlkvsdtkidsiedryprmltervreslriwkntekenatvahlvgalrscqmnlvad
lvqevqqardlqnrsgamspmswnsdastseas

20

128. 2e12 scFv-hIgG1 (SSS-P)H WCH2 WCH3-hFADD-TM/CT

Nucleotide sequence:

aagcttatggatttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgtcaccca
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaagtggtgaatattatgcacaagtta
25 atgcagtgttaccacagaaaccaggacagccacccaaactcctcatctctgtcgcacccaacgtagaatctggggtcctgccagg
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt
aggaaggttcttggacgttcggtggagggcaccaagctggaaatcaaacggggtggcggtggctcgggcggaggtgggtcgggt
ggcgggcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgacctct
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg
30 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaggacaactccaagagccaagtttctaaaaatgaa
cagctctgaaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagacctgaactcc
tgggtggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctccggagccctgaggtcacatgctggtggt
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcggtggaggtgcataatgccaagacaaagccgcg
35 ggaggagcagtaaacagcacgtacctgtgtgtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag
tgcaaggctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt
acacctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc
tctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
40 cactacacgcagaagagcctctccctgtctccgggtaagcgatccttgaacccgttctggtgctgtgcactcgggtgctgcca
gcctgtcgagcagcgagctgaccgagctcaagttcctatgcctcgggcgcgtgggcaagcgcaagctggagcgcgtgcagagcg
gcctagaccttcttccatgctgctggagcagaacgacctggagccccgggcacaccgagctcctgcgcgagctgctgcctccctg
cggcgccacgacctgctcggcgcgctgcagacttcaggcggggggcgccggccggggccgcgcctggggaagaagacctgt
gtgcagcatttaacgtcatatgtgataatgtggggaagattggagaaggctggctcgtcagctcaaaagtctcagacaccaagatcga
45 cagcatcgaggacagataacccccgaacctgacagagcgtgtcgggagtcactgagaatctggaagaacacagagaaggaga
acgcaacagtgggccacctggtgggggctctcaggtctgccagatgaacctggtggctgacctggtacaagaggttcagcaggc

ccgtgacctccagaacaggagtggggccatgtccccgatgtcatggaactcagacgcatctacctccgaagcgtcctgataactcga
gatcgataacaac

Amino acid sequence:

5 mdfqvqifsflisasvmsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
arfsrgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfggggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit
ctvsgfsltygvnwvrpppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqtdtdaryycardgysnfh
yyvmdywgqgtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwy
vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeitkn
10 qvsltclvkgyfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp
gkadpsnpflvllhsvssslssselteklfclgrvgkrklervqsgldlfsmlleqndlepghtellrellaslrhdllrrvddfeagaa
agaapgeedlcaafnvicdnvgkdwrrlarqlkvsdtkidsiedryprmltervreslriwkntekenatvahlvgalrscqmnlv
adlvqevqqardlqnrsgamspmswnsdastseas

15 129. 2e12 scFv-hIgG1 (SSS-P)H P238SCH2 WCH3-hFADD-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca
atctccagcttctttggctgtgtctctaggtcagagagccaccatctctcagagccagtgaaagtgtgaatattatgcacaagtta
atgcagtggtaccaacagaaaccaggacagccaccaaacctcctcatctctgctgcatccaacgtagaatctggggtccctgccagg
20 tttagtggcagtggtctgggacagacttcagcctcaatccatctgtggaggaggatgatattgaatgtattctgtcagcaaagt
aggaaggttccttgacgttcggtggagggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggt
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctggcggcctcacagagcctgtccatcacatgcaccgtct
cagggttctcattaaccggctatggtgtaaaactgggtcggcagcctccaggaaagggtctggagtggctgggaatgatatggggtg
atggaagcacagactataattcagcttcaaatccagactgagcatccaaggacaactccaagagccaagtttcttaaaaatgaa
25 cagtctgaaaactgatgacacagccagatactactgtgccagagatgggtatagtaactttcattactatgttatggactactgggtgcaa
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcc
tgggtggatcgtcagttctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgctggtggt
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag
30 tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctcaaagccaaaggcagccccgagaaccacaggtgt
acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgacatc
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaaaggatccttgaacccgttctggtgctgctgactcggtgtcgtcca
35 gcctgtcagcagcagctgaccgagctcaagttcctatgcctcgggcgcgtgggcaagcgcaagctggagcgcgtgcagagcg
gcctagaccttcttccatgctgctggagcagaacacctggagcccgggcacaccgagctcctgcgcgagctgctcgcctccctg
cggcgccacgacctgctgcggcgcgtcagcacttcgaggcgggggcggcggccggggccgcgcctggggaagaagacctgt
gtgcagcatttaacgtcatatgtgataatgtggggaagattggagaaggctggctcgtcagctcaaagtctcagacaccaagatcga
cagcatcgaggacagataacccccgcaacctgacagagcgtgtcggggagtcactgagaatctggaagaacacagagaaggaga
40 acgcaacagtgggccacctggtgggggtctcaggtctgccagatgaacctggtggctgacctggtacaagaggttcagcagggc
ccgtgacctccagaacaggagtggggccatgtccccgatgtcatggaactcagacgcatctacctccgaagcgtcctgataactcga
gatcgataacaac

45 Amino acid sequence:

mdfqvqifsflisasvimsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
 arfsgsgsgtdfslnihpveeddiamyfcqqsrvpwtfgggtkleikrgggsgggsgggsgqvlkesgpglvapsqslsit
 ctvsgfsltgygvnwvrpppgklewlgmiwgdgstdynsalksrslsitkdnksqvflkmnslqtddtaryycardgysnfh
 yyvmdywggqtsvtvssdlepkssdkthtspspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy
 5 vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgqprepvytlppsrdeltnk
 qvsltlvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp
 gkadpsnplvllhsvssslsssetelkflclgrvgkrklervqsgldlfsmlleqndlepghtellrellaslrhdllrrvddfeagaa
 agaapgeedlcaafnvidcnvgkdwrrlarqlkvsdtkidsiedryprnltervreslriwkntekenatvahlvgalrscqmnlv
 adlvqevqqardlqnrgamspmswnsdastseas

10

130. 1D8 scFv hIgG1 (SSS-P)H P238S CH2 WCH3-hCD80TMCT

Nucleotide sequence

aagcttatgattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gctccaacaaccatagctgcatctccaggaggagaaggtcaccatcacctgccgtgccagctccagttaagtacatgtactgttac
 15 cagcagaagtcaggcgcctccccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccatttactgtcagcagtgaggtagtactccgc
 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtggcggcgatctc
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 20 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaaatcaacagcttgcaactgat
 gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatctggagcccaa
 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatcgtcagttcttcttccccccaaaaccca
 aggacaccctcatgatctcccgaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagaccctgaggtcaagttcaac
 tggtagctggacggcgtggaggtgcataatgccaaagaccgcgggaggagcagtaaacagcacgtaccgtgtggtcagc
 25 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcga
 gaaaaccatctccaaagccaaaggcagccccgagaaccacaggtgtacaccctgccccatccgggatgagctgaccaagaa
 ccaggtcagcctgacctgctgtaaaaggcttctatccagcgacatcgccgtggagtgggagagcaatggcgagccggagaac
 aactacaagaccacgctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggacaagagcaggtggca
 gcagggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaa
 30 agcggatccttcgaacctgctcccatctgggccattacctaatactcagtaaatggaattttgtgatatgctgcctgacctactgctttg
 cccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgat

Amino acid sequence:

mdfqvqifsflisasvimsrgvdivltqspptiaaspgekvittrasssvsmywyqqksgasplwiydtsklasgvpnrfsg
 35 ssgtsyslaintmetedaatyycqqsstpltfsgstkleikrgggsgggsgggsgqvlkeagpglvqptqtlstctvsgfs
 ltsdgvhwirpppgklewmgiiydggtdynsaiksrslsrdtsksqvflkinslqtddtamyyccarihfdywgqgvmvtv
 ssdlepkssdkthtspspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree
 qynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgqprepvytlppsrdeltnkqvsltlvkgfypsdiav
 40 ewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgkadpsnllpswaitlis
 vngifvicltycfaprerrrrerlrresvrpv

131.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-hCD80TM/CT

Nucleotide sequence

45

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
 atctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaaagtt
 atgcagtggtagaacagaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctgggggtccctgccagg
 ttttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattctgtcagcaaagt
 5 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggtac
 cagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaataacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc
 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggggtcgggtggcggcgatctc
 10 aggtgcagctgaaggaggcaggacctggcctgggtgcaaccgacacagacctgtccctacatgactgtctctgggttctcattaa
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttctaaaaatcaacagctctgcaaacatgat
 gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa
 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggaccgtcagcttctcttccccccaaaacc
 15 aaggacacctcatgatctcccggaccttgaggtcacatgcgtgggtgggtgacgtgagccacgaagacctgaggtcaagttcaa
 ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtaaacagcacgtaccgtgtggtcag
 cgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccagccccatcg
 agaaaaccatctcacaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccgggatgagctgaccaaga
 accaggtcagcctgacctgcctgggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaa
 20 caactacaagaccacgcctcccgctgctggactccgacggctccttctcctctacagcaagctcaccgtggacaagagcaggtggc
 agcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggta
 aagcggatcctcgaacctgctcccatcctgggccattacctaatactcagtaaatggaatgttgatgctgcctgacctactgctt
 gccccaatgagcagagagagaaggaggaatgagagattgagaagggaaggtgtacgcctgtataaatcgat

25 Amino acid sequence:
 mdfqvqifsfllisasvimsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
 arfsgsgsgtdfslnihpveeddiamyfcqqsklmdfqvqifsfllisasvimsgvdivltqspttiaaspgekvtitcrassvsy
 mywyqqksgasplkiwydtsklasgvpnrfsgsgsgtsyslaintmetedaatyycqqwsstpltfsggtkleikrgggsggg
 gsgggsgsvqlkeagpqlvptqlsltctvsgfsltsdgvhwirppgkglewmgiyydgtdynsaiksrlsisrdsksqvf
 30 lkinslqtdtdamyycaihfdywggvmvtvssdlepkssdkthtsppspapellggspsvflfppkpkdtlmisrtpetcvv
 vdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgqpr
 epqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdsfflyskltvdkrsrwqqgnvfscsvm
 healhnhytqkslslspgkadsnllpswaitlisvngifviccltycfaprerrrrerlrrsvrp

35
132.1D8 scFv mIgAT4-hCD80TM/CT
 Nucleotide sequence

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggtac
 40 cagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaataacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc
 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtggcggcgatctc
 aggtgcagctgaaggaggcaggacctggcctgggtgcaaccgacacagacctgtccctacatgactgtctctgggttctcattaa
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 45 ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttctaaaaatcaacagctctgcaaacatgat
 gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatcacatctgttctc

Amino acid sequence:

mdfqvqifsfllisasvimsgvdivltqspttiaaspgekvtitcrasssvsmywyqqksgasplkiwydtsklasgvpnrfsg
sgsgtsyslaintmetedaatyycqqwsstpltfsgstkleikrgggsgggsgggsgggsgvqlkeagpglvqptqlslctvsgfs
ltsdgvhwirpppgklewmgiyydggt dynsaiksrlsirdtsksqvfllkinslqtdtdamyycaihfdywgqgmvtv
5 ssdhvcsrdftptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmvdldstasttqegelastqseltlsqkhwls
drtytcqvt yqghtfedstkkcadsnprgvsaylsrpsfdlfrksptitclvdlapskgtvnlwsrasgkpvnhstrkeekqm
gtltvtstlpvgtrdwiegetyqcrvthphlpralmrsttktsgraaapevyafatpewpgsrdkrtlacliqnfmpe disvqwlhn
evqlpdarhsttqprktksggffv fsrlevtraeweqkdeficravheaaspsqtvravsvnpgkadpsklpswaitlisvngifv
iccltycfaprcrerrrmerlrresvrpv

10

134.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mFADD-TM/CT

Nucleotide sequence

15 aagcttatggatttcaagtgcagatttgcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactggtag
cagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtgccagtg
ggcttgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc
tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcgatctc
aggtgcagctgaaggaggcaggacctggcctgggtgcaaccgacacagacctgtccctcacatgactgtctctgggttctcattaa
20 ccagcgtggtgtactggttcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttctaaaaatcaacagctctgcaactgat
gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcattggtcacagtctcctctgatctggagcccaa
atcttctgacaaaactcacatccccaccgtcccagcactgaactcctgggtggaccgtcagcttctcttccccccaaaaccca
aggacacctcatgatctcccgaccctgaggtcacatgcgtgggtggtagctgagccacgaagacctgaggtcaagttcaac
25 tggtagctggagggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtcagc
gtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggtctccaacaaagccctcccagccccatcga
gaaaacctctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaa
ccaggtcagcctgacctgctgggtcaaaaggcttctatccagcgacatcgccgtggagtgaggagcaatgggcagccggagaac
aactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaagtcaccgtggacaagagcaggtggca
30 gcagggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaacctacacgcagaagagcctctcctgtctccgggtaa
agcggatccttgaacatggaccattctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatctgatggagctcaa
gttcttgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacgggtgctgctggagcag
aacgacctggagcgcgggcacaccgggctgctgcgcgagtgctggcctcgtgcgccgacacgatctactgcagcgcctggac
gacttcgagggcggggacggcgaccgctgcgccccgggggagggcagatctgcaggtggcatttgacattgtgtgacaatgtgg
35 ggagagactggaaaagactggccgcgagctgaaggtgtctgagccaagatggatgggattgaggagaagtacccccgaagtc
tgagtgagcgggtaaggagagtgctgaaagtctggaagaatgctgagaagaagaacgcctcgggtggccggactggtcaaggcgc
tgcggacctgcaggtgaatctggtggctgacctggtggaagaagcccaggaatctgtgagcaagagtgagaatatgtcccagta
ctaagggttcaactgtgtcttctcagaaacacctgactcgagatcgat

40 Amino acid sequence:

mdfqvqifsfllisasvimsgvdivltqspttiaaspgekvtitcrasssvsmywyqqksgasplkiwydtsklasgvpnrfsg
sgsgtsyslaintmetedaatyycqqwsstpltfsgstkleikrgggsgggsgggsgggsgvqlkeagpglvqptqlslctvsgfs
ltsdgvhwirpppgklewmgiyydggt dynsaiksrlsirdtsksqvfllkinslqtdtdamyycaihfdywgqgmvtv
ssdlepkssdkthtspspapellggs vflfpkpkdltmisrtevtcvvvdvshedpevkfnwyvdgvevhnaktkpree
45 qynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgpprepqv tylppsrdeltknqvs lclvkgyfypsdiav
ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqksls lpsgkadpsnmdpflvllhs

lsgslsgndlmelkflcrervskrklervqsgldlftvllleqndlerghtglirellaslrhdllqrlddfeagtataappgeadlqvaf
divcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglvkalrtcrlnlvadlveeaquesvs
ksenmspvlrdstvsstetp

5 **135.1D8 scFv hIgG1 (SSS-P)H P238S CH2 WCH3-mFADD-TM/CT**

Nucleotide sequence

aagcttatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagtacatgtactggtac
cagcagaagtgcaggcgctcccctaaactctggatttatgacacatccaagctggcttctggaggtccaaatcgcttcagtggcagtg
10 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgtagtagtactccgc
tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcggcggtggtgggtcgggtggcgcggtatctc
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa
ccagcgatggtgtacactggattcgacagcctccaggaagggtctggaatggatgggaataatatattatgatggaggcacagatt
ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaaatcaacagctgcaaaactgat
15 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa
atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggtggatcgtcagcttctcttccccccaaaaccca
aggacacctcatgatctcccgaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaac
tggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagc
gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcagggtcctcaacaaagccctcccagccccatcga
20 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaa
ccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatggcgagccggagaac
aactacaagaccacgctcccgtgctggactccgacggctccttctctacagcaagctcaccgtggacaagagcaggtggca
gcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaa
agcggatccttgaacatggaccattctggtgctgctgcactgctgtccggcagcctgtcgggcaacgatctgatggagctcaa
25 gttcttgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacgggtgctgctggagcag
aacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgtcgcgccgacacgatctactgcagcgcctggac
gacttcgagggcggggacggcgaccgctgcggcccgggggaggcagatctgcaggtggcatttgacattgtgtgtgacaatgtgg
ggagagactggaaaagactggcccgagctgaaggtgtctgagccaagatggatgggattgaggagaagtacccccgaagtc
tgagtgcgagggttaaggagagctgaaagtctggaagaatgctgagaagaagaacgcctcgggtggccggactggtcaaggcgc
30 tgcggacctgcaggctgaatctggtggctgacctggtggaagaagccaggaatctgtgagcaagagtgagaatatgtccccagta
ctaagggatcaactgtgtcttctcagaaacacctgactcgagatcgat

Amino acid sequence:

mdfqvqifsfllisasvimsgvdivltqspttiaaspgekvitcrasssvsmywyqqksgasplwiydtsklasgvpnrfsg
35 ssgtsyslaintmetedaatyycqwsstpltfsgtkleikrgggsgggsgggsgvqlkeagpglvqptqlslctvsgfs
ltsdgvhwirppgkglewmgiiyydggttdynsaiksrslsrdtsksqvfkinslqtdtdamyyecarihfdywgqgvmvtv
ssdlepkssdkhtspspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree
qynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltknqvsltlvkgfypsdiav
ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgkadsnmdpflvllhs
40 lsgslsgndlmelkflcrervskrklervqsgldlftvllleqndlerghtglirellaslrhdllqrlddfeagtataappgeadlqvaf
divcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglvkalrtcrlnlvadlveeaquesvs
ksenmspvlrdstvsstetp

45 **136.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp3-TM/CT**

Nucleotide sequence:

aagcttatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagttacatgtactggtag
 cagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgtgccacttattactgtcagcagtgagtagtactccgc
 5 tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcggggcgggtgggtgggtcgggtggcggcgatctc
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa
 ccagcgatggtgtacactggattcgacagcctccaggaaaggggtctggaatggatgggaataatatattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaaatcaacagctctgcaaaactgat
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa
 10 atcttctgacaaaactcacatccccaccgtccccagcacctgaactcctgggtggaccgtcagcttctcttccccccaaaaccca
 aggacacctcatgatctccggaccttgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaac
 tggtagctggacggcggtggaggtgcataatgccaaagacaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagc
 gtctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccagccccatcga
 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaa
 15 ccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccgggagaac
 aactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggca
 gcagggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacagcagaagagcctctccctgtctccgggtaa
 agcggatccttgaacatggagaacaacaaaacctcagtggaattcaaaatccattaataattttgaagtaaagaccatacatgggagc
 aagtcagtgactctgggatctatctggacagtagttacaaaatggattatctgaaatgggcatatgcataataataataaagaact
 20 tccataagagcactggaatgtcatctcgtctggtacggatgtggacgcagccaacctcagagagacattcatgggctgaaataacc
 aagtcaggaataaaaaatgatcttactcgtgaagacattttgaattaatggatagtggttctaaaggaagatcatagcaaaaggagcagct
 ttgtgtgtgattctaagccatggtgatgaaggggtcatttatgggacaaatgggcctgttgaactgaaaaagttgactagcttctcag
 aggcgactactgccggagtctgactggaaagccgaaactcttcatcattcaggcctgccgggggtacggagctggactgtggcattga
 gacagacagtgaggactgatgaggagatggcttgcagaagataccggtggaggctgacttctgtatgcttactctacagcacctgg
 25 ttactattcctggagaaattcaaaggacgggtcgtggttcatccagtcctttgcagcatgctgaagctgtacgcgcacaagctagaatt
 tatgcacattctcactcgcgttaacaggaaggtggcaacggaattcagtccttctccctggactccactttccacgcaagaaacaga
 tcccggtgattgtgtccatgctcacgaaagaactgtactttatcactagctcgagatcgatg

Amino acid sequence;

30 mdfqvqifsflisavimsrgvdivltqspttiaaspgekvititrasssvsymywyqqksgasplwiydtsklasgvpnrfsg
 ssgtsyslaintmetedaatyccqwsstpltfsgstkleikrgggsgggsgggsgvqlkeagpglvqptqlslctvsgfs
 ltsdgvhwirppgkglewmgiiyydgtdynsaiksrlsirdtsksqvfkinslqtdtdamyycaarihfdywgqvmvtv
 ssdlepkssdkhtspspapellggpsvflfppkpkdltmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree
 35 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvylppsrdeltknqvsltclvkgyfypsdiav
 ewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslspsgkadpsnmennktsvd
 sksinnfevktihgksvdsigiylssykmdypemgiciniinnknfhkstgmssrsgtdvdaanlretfmglkyqvrnkndltr
 edilelmdsvskedhskrssfcvilshgdegviygtngpvelkkltsffrgdyrsltgkpklfiiqacrgtelcdgietsgtdede
 macqkipveadflyaystapgyyswrnskdgswfqslcsmlklyahklefmhiltrvnrkvatefesfslstdthakqkipciv
 40 smltkelyfyh

137.1D8 scFv hIgG1 (SSS-P)H P238S CH2 WCH3-mcasp3-TM/CT

Nucleotide sequence:

Aagcttatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagttacatgtactggtag
 45 cagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgtgccacttattactgtcagcagtgagtagtactccgc

tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatc
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcatgactgtctctgggttctcattaa
 ccagcgtatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttcttaaaaatcaacagtctgcaaactgat
 5 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtcacagtctcctctga

Amino acid sequence:

mdfvqvifsfllisasvimsrgvdivltqspttiaaspgekvitcrasssvsymywyqqksgasplwiydtsklasgvpnrfsg
 sgsdgsyslaintmetedaatyycqqwsstpltfsggtkleikrgggsgggsgggsgqvqlkeagpqlvqptqlstctvsfsgs
 10 ltsdgvhwirpppgklewmgiyydggttdynsaiksrslsrdtsksqvflikslqtdtdamyycarihfdywggqvmvvtv
 ssdlepkssdkthtspspapellggssvflfppkpkdtlmistrpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree
 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeitknqvsitclvkgyfypsdiav
 ewesngqpennyktppvldsdsfflyskltvdksrwqqgnvfscsvmhleahnytkqslslspgkadspsnsmennkts
 vdsksinnfevktihgsksvdsgiyldssykmdypemgicliinnknfhkstmssrsrgtdvdaanlretfmgikyqvrnknd
 15 ltredilelmdsvskedhskrssfvcvilshgdegviygtngpvelkkltstffrgdycrsltgkpklfiiqacrgtelcdgietdsgtde
 emacqkipveadflyaystapgyyswrnskdgsfwfqlscsmklyahklefmhiltrvnrkvatefesfslstdfhakkqipci
 vsmlltkelyfyh

138.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp8-TM/CT

20 Nucleotide sequence

aagcttatgattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggtac
 cagcagaagtcaggcgctcccctaaactctggattatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccatttactgtcagcagtgtagtagtactccgc
 25 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatc
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcatgactgtctctgggttctcattaa
 ccagcgtatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttcttaaaaatcaacagtctgcaaactgat
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtcacagtctcctctgatctggagcccaa
 30 atcttctgacaaaactcacatccccaccgtcccagcactgaactcctgggtggaccgtcagtttctcttccccccaaaaccca
 aggacacctcatgatctcccgaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaac
 tggtagctggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtcagc
 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcga
 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaa
 35 ccaggtcagcctgacctgctggtcaaaaggtctatcccagcgacatcgccgtggagtgaggagcaatgggcagccgggagaac
 aactacaagaccacgcctcccgtgctggactccgacggctccttctctacagcaagctcaccgtggacaagagcaggtggca
 gcaggggaacgtcttctcatgctccgtgatgcagtgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaa
 agcggatccttgaacatggattccagagttgtctttatgctattgctgaagaactgggcagtgagacctggctgcctcaagttcct
 gtgcttggactacatcccacacaagaagcaggagaccatcgaggatgccagaagctatttctgagggtcggggaaaaggggatgt
 40 tggaggaaggcaatctgtcttctgaaagagctgctttccacatcagtcgggtgggacctgctggtcaacttcttagactgcaaccga
 gaggagatggtgagagagctgcgggatccagacaatgccagatttctcctacagggtcatgctcttaagctctcagaagaagt
 agcgagttggaattgagatctttaagttcctttgaacaatgagatccccaaatgaagctggaagatgactgagcctgcttgaatttt
 ttagaagtgaggaagaggacatgctggcagaaaataacttgaaaccctaaaaatcaatctgtgaccaggtcaacaagagcctgct
 ggggaagatcgaggattatgaaagatcaagcacagagagaagaatgagccttgaagggaagggaagagttgccaccttcagtttgg
 45 atgagatgagcctcaaaatggcggaactgtgtgactgccaagagaacaagacagtgagtcacggacttcagacaaagtttaccaa
 atgaagaacaaacctggggatactgtctgatcatcaacaatcatgatttcagcaaggccgggaagacataacccaactccgaaaa

atgaaggacagaaaaggaacagactgtgataaagaggctctgagtaagaccttaaggagcttcattttgagatagtatcttacgacg
actgcactgcaaatgaaatccacgagattctagaaggctaccaagcgcagaccacaagaacaaagactgcttcactgctgtatcct
atcccacggtgacaagggtgtcgtctatggaacggatgggaaggaggcctccatctatgacctgacatcttacttcactggttcaagt
gccccttccctgtctgggaaacccaagatcttttattcaggcttgccaaggaaagtaactccagaaaggagtgcctgatgaggcagg
5 cttcagcaacagaaccacactttagaagtggattcatcatctcacaagaactatattccggatgaggcagactttctgctgggaatgg
ctacggtgaagaactgcgttctaccgagatcctgtgaatggaacctggtatattcagtcactttgccagagcctgagggaagatgt
cctcaaggagatgacattcttagcatcctgactggcgtgaactatgacgtgagcaataaagacgacaggaggaacaagggaagca
gatgccacagcccaccttcacactacggaagaagctcttctccctccctaatgactcgagatcgatt

10 Amino acid sequence

mdfqvqifsflisasvimsgvdivltqspttiaaspgekvitcrasssvsymywyqqksgasplwiydtsklasgvpnrfsg
sgsgtsyslaintmetedaatyycqqwsstpltfsgstkleikrgggsgsgsgsgsgsqvqlkeagpglvqptqtlstctvsfgs
ltsdgvhwirppgkglewmgiyydggt dynsaiksrlsisrdsksqvflkinslqtdtdamyycarihfdywgqgmvtv
ssdlepkssdkthtspspapellgpgsvflfppkpkdtlmisrtevtcvvvdvshedpevkfnwyvdgvevhnaktkpre
15 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsltclvkgyfypsdiav
ewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhleahnytkslslspgkadsnmdfqsclyai
aeelgsedlaalkflclidyphkkqetiedaqklflrlekglmleegnlsflkellfhisrwdllvnfldcnreemvrelrdpdnaqis
pyrvmlfklseevselelrsfkflnneipkckleddlsleifvemekrtmlaennletlksicdqvnskllgkiederssterms
legreelppsvldemslkmaelcdspreqdsesrtdkvyqmknkprgycliinnhdfskareditqlrkmkdrkgtdcdkeal
20 sktfkelhfeivsyddctaneiheilegyqsadhknkdcficcilshgdkgvvygtdgkeasiydltsyftgskcpslsgkpkiffi
qacqgsnfqkgvpdeagfeqqnhtlevdssshknyipdeadflgmatvkncvsyrdpvngtwiyqslcqlsrercpqgddil
siltgvnydvsnkddrmkgkqmpqptflrklkffpp

139.1D8 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-mcasp8-TM/CT

25 Nucleotide sequence

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactggtac
cagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
ggctctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc
30 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcgatctc
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgactgtctctgggttctcattaa
ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagtttcttaaaaaatcaacagctctgaaactgat
gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcattggtcacagtctcctctgatctggagcccaa
35 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggtggatcgtcagtcctctctccccccaaaacca
aggacacctcatgatctcccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtaagttcaac
tggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtcagc
gtctcaccgtcctgcaccaggactggctgaatggcaaggagtagaagtgaaggtctccaacaaagccctccagcccccatcga
gaaaccatctccaagccaagggcagccccgagaaccacaggtgtacacctgcccccatccgggatgagctgaccaagaa
40 ccaggtcagcctgacctgctgtgtaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccgggagaac
aactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggca
gcagggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaa
agcggatcctcgaacatggattccagagttgtcttatgctattgctgaagaactgggcagtgaagacctggctgcctcaagttcct
gtgcttgactacatcccacacaagaagcaggagaccatcgaggatgccagaagctatttctgaggctgcgggaaaaggggatgt
45 tggaggaaggcaatctgtcttctgaaagagctgctttccacatcagtcgggtgggacctgctggtcaacttcctagactgcaaccga
gaggagatggtgagagagctgcgggatccagacaatgccagatttctcctacagggtcatgctcttaagctctcagaagaagtg

agcgagttggaattgagatcttttaagttcctttgaacaatgagatcccaaatgtaagctggaagatgacttgagcctgcttgaaat
 ttagaaatggagaaggaccatgctggcagaaaataacttgaaaccctaaatcaatctgtgaccaggtaacaagagcctgct
 ggggaagatcgaggattatgaaagatcaagcacagagagaagaatgagccttgaaggagggaagagttgccaccttcagtttgg
 atgagatgagcctcaaatggcggaactgtgtgactcgccaagagaacaagacagtgagtcacggacttcagacaaagttacaa
 5 atgaagaacaaacctcggggatactgtctgatcatcaacaatcatgatttcagcaaggcccggaagacataaccaactccgaaa
 atgaaggacagaaaaggacagactgtgataaaggagctctgagtaagacctttaaggagcttcatttgagatagtatcttacgacg
 actgcactgcaaatgaaatccacgagattctagaaggctaccaaaagcgcagaccacaagaacaagactgttcctctgtgtatcct
 atccacgggtgacaagggtgtcgtctatggaacggatgggaaggaggcctccatctatgacctgacatcttacttcactgttcaaagt
 gcccttcctgtctgggaaaccaagatcttttcattcaggcttgccaaggaaagtaactccagaaaggagtgccatgagggcagg
 10 cttcagcaacagaaccacacttagaagtggattcatctcacagaactatattccgatgaggcagactttctgtgggaatgg
 ctacggatgaagaactgcgttccctaccgagatcctgtgaatggaacctggtatattcagtcactttgccagagcctgagggaagatgt
 cctcaaggagatgacattcttagcatcctgactggcgtgaactatgacgtgagcaataaagacgacaggagggaacaagggaagca
 gatgccacagcccaccttcacactacggaagaagctcttctccctccctaatgactcgagatcgattc

15 Amino acid sequence

mdfqvqifsflisavimsrgvdivltqspttiaaspgekvitcrasssvsymywyqqksgasplwiydtsklasgvpnrfsg
 sgsgtsyslaintmetedaatyccqwsstpltfsgtkleikrgggsgggsgggsgqvlkeagpglvqptqlslctvsgfs
 ltsdgvhwirppgkglewmgiyydggt dynsaiksrlsirdtsksqvlkinslqtdtdamyycaihfdywggvmvtv
 ssdlepkssdkthtspspapellggssvflfpkpkdltmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree
 20 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsltclvkgyfypsdiav
 ewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgkadsnmdfqsclyai
 aeelgsedlaalkflclidyphkkqetiedaqklflrrekgmleegnlsfkllelfnhisrwdllvnfldcnreemvrelrdpnaqis
 pyrvmlfklseevselelrsfklfnneipkckleddlsleifvemekrtmlaennletlksicdqnksllgkiedyerssterms
 legreelppsvldemslkmaelcdspreqdsesrtdskvyqmknkprgycliinnhdfskareditqlrkmkdrkgtdcdkeal
 25 sktfkelhfeivsyddctaneiheilegyqsadhknkdcfcicilshgdkgvvygtdgkeasiydltsyftgskcpslsgkpkiffi
 qacqgsnfqkgvpdeagfeqqnhtlevdssshknyipdeadflgmatvkncvsyrpvngrtwiyqlscqlsrercpqqddil
 siltgvnydvsnkddrrnkkgkmpqptflrklffpp

140.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcasp3-TM/CT

30 aagcttatgattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactgtgac
 cagcagaagtcaggcgctccctccctaaactctggaattatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgtgccacttattactgtcagcagtgaggtagtactccgc
 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcggcggtgggtgggtggcgcgatctc
 35 aggtgcagctgaaggaggcaggacctggcctggtgaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagtttcttaaaaatcaacagctctgcaaactgat
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa
 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggtggaccgtcagttctcttccccccaaaaccca
 40 aggacacctcatgatctcccggaccctgaggtcatatgcgtggtggagctgagccacgaagacctgaggtcaagttcaac
 tggtagctggacggcgtggaggtgcataatgccaaagaccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagc
 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaagccctccagcccccatcga
 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatcccgggatgagtgaccaagaa
 ccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaac
 45 aactacaagaccacgctcccgtgctggactccgacggctccttctctacagcaagctcaccgtggacaagagcaggtggca
 gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaa

agcggatccttgaacatggagaacactgaaaactcagtgattcaaaatccattaaaaatttgaaccaagatcatacatggaagc
 gaatcaatggactctggaatatccctggacaacagttataaatggattatcctgagatgggtttatgtataataattaataagaatttt
 cataaaagcactggaatgacatctcggctcgtgtacagatgtcgcagcagaaacctcagggaaacattcagaaactgaaatatgaag
 tcaggaataaaaatgatcttacacgtgaagaaattgtggaattgatgcgtgatgtttctaaagaagatcacagcaaaaggagcagtttg
 5 tttgtgtgcttctgagccatggatgaagaagggaataattttggaacaaatggacctgttgacctgaaaaaataacaaacttttcagagg
 ggatcgtttagaagttaactggaaaacccaaacttttcattattcaggcctgccgtggtacagaactggactgtggcattgagacag
 acagtgggtgtgatgatgacatggcgtgtcataaaataaccagtggaggccgacttctgtatgcatactccacagcacctggttattatc
 ttggcgaaattcaaaggatggctcctggttcacagtcgctttgtccatgctgaaacagtatgccgacaagctgaatttatgcacatt
 cttacccgggttaaccgaaaggtggcaacagaatttgagtccttttcctttgacgctactttcatgcaaaagaaacagattccatgtattgt
 10 tccatgctcacaagaactctattttatcactaactcagatcgata

Amino acid sequence:

mdfqvqifsllisasvmsrgvdivltqspttiaaspgekvtitcrasssvsymywyqqksgasplwiydtsklasgvpnrfsg
 sgshtsyslaintmetedaatyccqwsstpltfsgstkleikrgggsgggsgggsgqvlkeagplvqptqlslctvsgfs
 15 ltsdgvhwirpppgklewmgiyydggttdynsaiksrlsrdtsksqvlkinslqddtamyycaarihfdywgqvmvtv
 ssdlepkssdkthtspspapellggpsvflfppkpdltmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree
 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvylppsrdeltnqvsiltclvkgyfypsdiav
 ewesngqpennykthpvltdsgsflyskltvdksrwqqgnvfscsvmhhealhnhytqkslslspgkadsnmentensvd
 sksiknlepkiihgsemdsgisldnsykmdypemglciinnknfhkstgmtsrsgtdvdaanlretfmkyevmknldtre
 20 eivelmrdvskedhskrssfvcvllshgeegifgtngpvdllkitnffrgdrclstgkpklfiiqacrgtelcgiestdsgvdddm
 achkipveadflyaystapgyyswrnskdgswfqslcamlkqyadklefmhiltrvnrkvatfefsfdatfhakkqipcivs
 mltkelyfyh

141. 1D8 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp3-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtctccaacaaccatagctgcattctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactggtagc
 cagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc
 30 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcggcggtggtgggtcgggtggcggcgatctc
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa
 ccagcgatgggtgtacactggattcgacagcctccaggaaagggcttggaatggatgggaataatataattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagctgcaaaactgat
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa
 35 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggtggatcgtcagtccttcttccccccaaaaccca
 aggacaccctcatgatctcccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagaccctgaggtcaagttcaac
 tggtagctggacggcgtggaggtgcataatgccaagacaagccgcgggaggagcagtaaacagcacgtaccgtgtggtcagc
 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggtctccaacaagccctccagcccccatcga
 gaaaaccatctccaaagccaagggcagccccgagaaccacaggtgtacacacctgcccccatccgggatgagctgaccaagaa
 40 ccaggtcagcctgacctgcctgggtcaaaggcttctatccagcgacatcgccgtggagtgaggagcaaatgggcagccgggagaac
 aactacaagaccagcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggca
 gcaggggaacgtcttctcatgctccgtgatgaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggttaa
 agcggatccttgaacatggagaacactgaaaactcagtgattcaaaatccattaaaaatttgaaccaagatcatacatggaagc
 gaatcaatggactctggaatatccctggacaacagttataaatggattatcctgagatgggtttatgtataataattaataagaatttt
 45 cataaaagcactggaatgacatctcggctcgtgtacagatgtcgcagcagaaacctcagggaaacattcagaaactgaaatatgaag
 tcaggaataaaaatgatcttacacgtgaagaaattgtggaattgatgcgtgatgtttctaaagaagatcacagcaaaaggagcagtttg

5 tttgtgtccttctgagccatgggtgaagaaggaataatTTTTGGAACAAATGGACCTGTTGACCTGAAAAAATAACAACTTTTCAGAGG
 ggatcgtttagaagtctaactggaaaacccaaacttttcattattcaggcctgccgtggtacagaactggactgtggcattgagacag
 acagtgggtgtgatgatgacatggcgtgtcataaaataaccagtggaggccgacttctgtatgcataccacagcacctgggtattattc
 ttggcgaaattcaaaggatggctcctggttcatccagtcgctttgtgcatgctgaaacagtatgccgacaagcttgaaattatgcacatt
 cttacccgggttaaccgaaaggtggcaacagaatttgagtcttttctttgacgctacttttcatgcaaagaaacagattccatgtattgt
 ttccatgctcacaaaagaactctattttatcactaactcgagatcgataa

Amino acid sequence:

10 mdfqvqifsfllisasvimsrgvdivltqspttiaaspgekvitcrasssvsymywyqqksgasplkiwydtsklasgvpnrfsg
 ssgtsyslaintmetedaatyycqqsstpltfsgsgtkleikrgggsgggsgggsgqvlkeagpglvqptqlslctvsgfs
 ltsdgvhwirpppgklewmgiiyydgtdynsaiksrslsrdtsksqvlkinslqtdtdamyycaihfdywggqvmvttv
 ssdlepkssdkthtspspapellggssvflfppkpkdtlmisrtevtcvvvdvshedpevkfnwyvdgvevhnaktkpre
 qynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsllclvkgfypsdiav
 ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhhealhnhytqkslslspgkadsnmentensvd
 15 sksiknlepkiihgsesmdsgisldnsykmdypemglciinnknfhkstgmtsrsgtdvdaanlretfnlkyevmknldtre
 eivelmrdvskedhskrssfvcvllshgeegiifgtngpvdllkitnffrgdrsrsltgkpklfiiqacrgtelcgtetdsgvdddm
 achkipveadflyaystapgyyswrnskdgswfiqslcamlkqyadklefihlitrnrvkatefesfsdatfhakkqipcivs
 mltkelyfyh

20

142.1D8 scFv hIgG1 (SSS-S)H WCH2 WCH3-hcasp8-TM/CT

Nucleotide sequence:

aagcttatgattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtcaccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagtacatgtactggtac
 25 cagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc
 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgtggctcgggcgggtggtgggtcgggtggcggcgatctc
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa
 ccagcgatggtgtacactggattcgacagcctccaggaaagggctggaatggatgggaataatatattatgatggaggcacagatt
 30 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaaataacagctgcaaaactgat
 gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa
 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggtggaccgtcagttctctctccccccaaaaccca
 aggacaccctcatgatctcccgaccctgaggtcatatgcgtggtggacgtgagccacgaagaccctgagggtcaagttcaac
 tggtagctggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtcagc
 35 gtctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagcccccatcga
 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccggtatgagctgaccaagaa
 ccaggtcagcctgacctgctgtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccgggagaac
 aactacaagaccacgctccgtgctggactccgacggctccttctcttacagcaagctcaccgtggacaagagcaggtggca
 gcagggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaa
 40 agcggatccttcgaacatggacttcagcagaaatctttatgatattggggaacaactggacagtgaagatctggcctcctcaagttcc
 tgagcctggactacattccgaaaggaaagcaagaacccatcaaggatgccttgatgtattccagagactccaggaaaagagaatgt
 tggaggaaagcaatctgtccttctgaaggagctgctctccgaattaatagactggatttctgattacctacctaactagaaagg
 aggagatggaaagggaacttcagacaccaggcagggtcctcaattctgcctacagggtcatgctctatcagatttcagaagaagtga
 gcagatcagaattgaggtctttaagttcttttgaagaggaaatctccaaatgcaaaactggatgatgacatgaacctgctggatatttc
 45 atagagatggagaagagggtcatcctgggagaaggaaagttggacatcctgaaaagagtcctgtgccccaatcaacaagagcctgct
 gaagataatcaacgactatgaagaattcagcaaaagagagaagcagcagcctgaagggaagtcctgatgaattttcaaatggggagg

agttgtgtggggaatgacaatctcggactctccaagagaacaggatagtgatcacagactttggacaaagtttaccaaatgaaaag
 caaacctcggggatactgtctgatcatcaacaatcacaattttgcaaaagcacgggagaaagtgccaaacttcacagcattagga
 caggaatggaacacacttgatgcaggggctttgaccacgacctttgaagagcttcattttgatcaagccccacgatgactgcaca
 gtagagcaaactatgagattttgaaaatctaccaactcatggaccacagtaacatggactgctcatctgctgtatcctctcccatggag
 5 acaagggcacatctatggcactgatggacaggagggcccccacatctatgagctgacatctcagttcactggtttgaagtgcccttcctt
 gctggaaaacccaaagtgtttttattcaggctgtcagggggataactaccagaaggtatacctgttgagactgattcagaggagca
 accctatttagaaatggatttatcatcacctcaaacgagatatatcccgatgaggctgactttctgctggggatggccactgtgaataa
 ctgtgtttcctaccgaaacctgcagagggaacctggtacatccagtcactttgccagagcctgagagagcgtgctcctcaggcgga
 tgatattctaccatcctgactgaagtgaactatgaagtaagcaacaaggatgacaagaaaaacatggggaaacagatgcctcagcc
 10 tactttcacactaagaaaaaaacttgcttcccttctgattgagcatgcatcgata

Amino acid sequence:

mdfqvqifsfllisasvmsrgvdivltqspttiaaspgekvitcrasssvsmywyqqksgasplwiydtsklasgvpnrfsg
 15 ssgtsyslaintmetedaatyccqwsstpltfsgtkleikrgggsgggsgggsgvqlkeagpglvqptqtlstctvsfgs
 ltsdgvhwirpppgklewmgiiyydggtynsaiksrlsirdtsksqvfkinslqtdtdamyyarihfdywgqgmvtv
 ssdlepkssdkthtspspapellggsplfpkpkdltmisrtpevtcvvdvshedpevkfnwyvdgvevhnaktkpree
 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvylppsrdeltknqvsltclvkgyfypsdiav
 ewesngqpennykthpvltdsgsflyskltvdksrwqqgnvfscsvmhleahnytkslslspgkadsnmdfsrnllydi
 20 geqldsedlaslkflsldyipqrkqepikdalmlfqlrlekrmlleenslflkellfrinrldlitylntrkeemerelqtpraqisay
 rvmlyqiseevsrslrsfklqeeiskckldddmnlldifiemekrvilgegkldilkrvcaqinkslkiindyeefskersssle
 gspdefsngeelcgvmtisdspreqdsesqtdkvyqmkskprgycliinnhnfakarekvpklhsirdnrnthldagaltttfee
 lhfeikphddctveqiyelkiyqlmdhsnmdcficilshgdkgiiygdgqeapiyeltsqftglkcpslagkpkvffiqacqg
 dnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnvcvsyrnpaegtwyiqslcqlsrercprgddiltitvevny
 25 evsnkddkknmgkqmpqptflrkkllvpsd

143.1D8 scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hcasp8-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 30 gctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactggtag
 cagcagaagtcaggcgccctcccctaaactctggatttatgacacatcaagctggcttctggagttccaaatcgcttcagtggcagt
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccattattactgtcagcagtgaggtagtactccgc
 tcacgttcgggtctgggaccaagctggagatcaaacgggtggcgggtggctcgggcgggtggtgggtcgggtggcgccgatctc
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa
 35 ccagcgatggtgtacactggattcagacgctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaaatcaacagctctgcaaaactgat
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa
 atcttctgacaaaactcacacatccccaccgtcccagcacctgaactcctgggtggatcgtcagttctcttccccccaaaaccca
 aggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtagcgtgagccacgaagacctgaggtaagttcaac
 40 tggtagctggcaggcgtggaggtgcataatgccaaagacaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagc
 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcga
 gaaaaccatctccaaagccaaggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaa
 ccaggctcagcctgacctgcctggtcaaaggcttctatccagcgacatcgccgtggagtgaggagagcaatgggcagccgggagaac
 aactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggca
 45 gcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggtaa
 agcggatcctcgaacatggacttcagcagaaatcttatgatattggggaacaactggacagtgaagatctggcctccctcaagttcc

5 tgagcctggactacattccgcaaaggaagcaagaacccatcaaggatgccttgatgtattccagagactccaggaaaagagaatgt
 tggaggaaagcaatctgtccttcctgaaggagctgctctccgaattaatagactggatttgctgattacctacctaactagaaagg
 aggagatggaaagggaacttcagacaccaggcagggtcaaattctgcctacagggtcatgctctatcagattcagaagaagtga
 gcagatcagaattgaggtcttttaagtttctttgcaaggaggaaatccaaatgaaaactggatgatgacatgaacctgctggatatttc
 10 atagagatggagaagagggtcatcctgggagaaggaaagtggacatcctgaaaagagtctgtgccaaatcaacaagagcctgtg
 gaagataatcaacgactatgaagaattcagcaaagagagaagcagcagcctgaaggaaagtcctgatgaattttcaaatggggagg
 agttgtgtggggaatgacaatctcggactctccaagagaacaggatagtgaatcacagactttggacaaagttaccaaatgaaaag
 caaacctcggggatactgtctgatcatcaacaatcacaattttgcaaaagcacgggagaaagtgccaaacttcacagcattaggga
 caggaatggaacacacttgatgcaggggctttgaccacgacctttgaagagcttcatttgagatcaagcccacgatgactgcaca
 15 gtagagcaaactctatgagatttgaaaatctaccaactcatggaccacagtaacatggactgcttcactctgctgtatcctctccatggag
 acaagggtcatctatggcactgatggacaggagcccccatctatgagctgacatctcagttcactggttgaaagtgcccttcctt
 gctggaaaacccaaagtgtttttattcaggctgtcagggggataactaccagaaaggtatacctgttgagactgattcagaggagca
 accctatttagaaatggatttatcatcacctcaaacgagatatatccggatgaggctgactttctgctggggatggccactgtgaataa
 ctgtgttctaccgaaacctgcagagggaacctgtacatccagtcactttgccagagcctgagagagcagatgtcctcagggcga
 20 tgatattctaccatcctgactgaagtgaactatgaagtaagcaacaaggatgacaagaaaacatggggaaacagatgcctcagcc
 tactttcacactaagaaaaaactgtcttccttctgattgagcatgcatcgataa

Amino acid sequence:

20 mdfqvqifsfllisasvmsrgvdivltqspttiaaspgekvtitrasssvsymywyqqksgasplwiydtsklasgvpnrfsg
 sgsgtsyslaintmetedaatyycqqsstpltfsgstkleikrgggsgggsgggsgvqlkeagpglvqptqtlslctvsgfs
 ltsdgvhwirpppgklewmgiiyydgtdynsaiksrlsirdtsksqvlkinslqtdtdamyycaihfdywgqgvmvtv
 ssdlepkssdkthtspspapellggssvflfppkpkdtlmisrtevtcvvvdvshedpevkfnwyvdgvevhnaktkpre
 25 qynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrdeltknqvsitclvkgyfypsdiav
 ewesngqpennykttpvldsdsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslspsgkadsnmdfsmlydi
 geqlsdslaskflsldyipqrkqepikdalmlfqlrlekrmlleenslsfkellfrinrldlitylntrkeemerelqtpraqisay
 rvmlyqiseevsrsefsfllqeeiskckldddmnlldifiemekrvilgegkldilkrvcaqinksllkiindyefskerssle
 gspdefsngeelcgvmtisdspreqdsesqldkvyqmkskprgycliinnhnfakarekvpklhsirdrnghldagalttfee
 30 lhfeikphddctveqiyelkiyqlmdhsnmdecficclshgdkgiyytdgqeapiyeltsqftglkcpslagkpkvffiqacqg
 dnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnvsvyrnpaegtwyiqslcqlsrercprgddilttevnv
 evsnkddkknmgkmpqptflrkklvfspd

144.

35 **145. hCTLA4 IgAH IgACH2CH3**

Nucleotide sequence:

40 atggcttgcccttgatttcagcggcacaaggctcagctgaacctggctgccaggacctggccctgcactctcctgtttttcttcttcat
 ccctgtcttctgcaaagcaatgcacgtggccagcctgctgtgtgactggccagcagccaggcctcgcagcttctgtgtgagat
 gcatctccaggcaaaagccactgaggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtcgggcaacct
 45 acatgacggggaatgagttgaccttctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactatccaagg
 actgagggccatggacacgggactctacatctgaagggtgagctcatgtaccaccgccatactacctgggcataggcaacggaa
 ccagatttatgtaattgatccagaacctgtcccagattctgatcagccagttccctcaactccactaccccatctccctcaactccac
 ctaccccatctccctcatgctgccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcagcct
 cacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgtgttcaaggac
 50 cacctgaccgtgacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagacctca
 cttgcatgctgcctaccccgagtccaagaccccgctaaccgccacctctcaaaatccggaaacacattccggcccgaggtccac

ctgctgccgccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatg
 tgctggttcgctgggtgcaggggtcacaggagctgccccgcgagaagtacgtgactgggcatcccgaggagcccagccagg
 gcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggg
 ccacgaggccctgccgtggccttcacacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgtgtcat
 5 ggcggagggtggacggcacctgctactgataatctaga

Amino acid sequence:

maclgfqrhkaqlnlaartwpctllffllfipvfckamhvaqpavvlassrgiasfvceyaspkgatevrvtlrqadsqvtevc
 tymtgneltfddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyyidpepcpdsdqpvpstptps
 10 pstptpspscchprlsihrpaledlllgseailtctltglrdasgvtftwtpssgksavqgppdrdlcgcysvssvlpgaepwnh
 ktftctaaypesktpilatlsksgntfrpevhllpppseelalnelvtltclargfspkdvlvrlqgsqelprekyltwasrqepsqg
 ttfavtsilrvaaedwkkgdtdfscmvghealplafktidrlagkpthvnsvvmaevdgtcy

146. hCTLA4 IgA WH WCH2 T4CH3 (hCTLA4 IgAH IgACH2CH3)

15 Nucleotide sequence:

atggcttgccttggatttcagcggcacaaggctcagctgaacctggctgccaggacctggccctgactctcctgtttttcttcttcat
 ccctgtcttctgcaaagcaatgcacgtggccagcctgctgtgtactggccagcagccgaggcatcggcagcttctgtgtgagtat
 gcatctccaggcaaagccactgaggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtcggcaacct
 acatgacggggaatgagttgaccttctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaagg
 20 actgagggccatggacacgggactctacatctgcaagggtggagctcatgtaccaccgccatactacctgggcataggcaacggaa
 ccagatttatgtaattgatccagaaccgtgccagattctgatcagccagttccctcaactccacctaccccatctccctcaactccac
 ctaccccatctccctcatgctgccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcct
 cacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggac
 cacctgaccgtgacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttca
 25 ctgactgctgcctacccgagtcgaagacccccgctaaccgccacctctcaaaatccggaaacacattccggcccgaggtccac
 ctgctgccgccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatg
 tgctggttcgctgggtgcaggggtcacaggagctgccccgcgagaagtacgtgactgggcatcccgaggagcccagccagg
 gcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggg
 ccacgaggccctgccgtggccttcacacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgtgtcat
 30 ggcggagggtggactgataatctaga

Amino acid sequence:

MacLgfqrhkaqlnlaartwpctllffllfipvfckamhvaqpavvlassrgiasfvceyaspkgatevrvtlrqadsqvtevc
 atymtgneltfddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyyidpepcpdsdqpvpstptp
 35 spstptpspscchprlsihrpaledlllgseailtctltglrdasgvtftwtpssgksavqgppdrdlcgcysvssvlpgaepwnh
 gktftctaaypesktpilatlsksgntfrpevhllpppseelalnelvtltclargfspkdvlvrlqgsqelprekyltwasrqepsq
 gtttfavtsilrvaaedwkkgdtdfscmvghealplafktidrlagkpthvnsvvmaevd

147. hIGA WH WCH2 T18CH3

40 Nucleotide sequence:

Tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcac
 tgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggt
 gtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctgtggtgctacagcgtgtcc
 agtgcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgcactgctgcctacccgagtcgaagacccccgct
 45 aaccgccacctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccctgaac
 gagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctgggtgcaggggtcacaggagctgc

cccgcgagaagtacgtgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgt
ggcagccgaggactggaagaagggggacaccttctcgtcatggtgggccacgaggccctgccgctggccttcacacagaagac
catcgaccgcttggcgggtaaatgataatctaga

5 Amino acid sequence:

dqvpvstptpsptpspspcchprlslhrpaledlllgseailtctltglrdasgvtftwtpssgksavqgppdrdlcgcysvssv
lpgcaepwnhgktfictaaypesktpatlsksngtfrpevhllpppseelalnelvtltclargfspkdvlvrwlqgsqelprekyl
twasrqepsqgtttfavtsilrvaaedwkkgdtfscmvghealplafqtidrlagk

10 148. 2h7 scFv IgA WH WCH2 T4CH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg
taccagcagaagccaggatcctcccccacccctggatttatgcccacccaacctggcttctggagtcctgctcgttcagtgga
15 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc
tctcaggttatctacagcagctctggggctgagctggtaggcctggggcctcagtgaaagatgctcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggatggagctatttatccaggaaatggtgatact
tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
20 atctgaagactctgcggctctatttctgtgcaagagtgggtactatagtaacttctactggtacttcgatgtctggggcacagggaccac
ggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgtgccac
ccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag
agatgcctcaggtgtcaccttcacctggagccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctg
ctacagcgtgtccagtgctcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgtgcctaccccgagtc
25 caagaccccgttaaccgccaccctctcaaaatccggaacacattccgccccgaggtccacctgctgccgccgccgtcgaggagg
ctggccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgtggttcgctggctgcaggggtc
acaggagctgccccgcgagaagtacgtgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccag
catactgcgcgtggcagccgaggactggaagaagggggacaccttctcgtcatggtgggccacgaggccctgccgctggccttc
acacagaagaccatcgaccgttggcgggtaaacccacccatgtcaatgtgtctgttgcacggagggtgactgataatctaga

30

Amino acid sequence:

mdfqvqifslilasviiargqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg
sgsgtsysltisrveadaatyycqqsfnpptfgagtkleldggsgggsgggssqaylqqsgaelvrpgasvkmsckas
gytftsynmhwwkqtrqglewigaiypngdtsynqkfkgkatltvdksstaymqlssltedsavyfcarvvyysnsywy
35 fdvwtgtttvtvssdqvpstptpsptpspspcchprlslhrpaledlllgseailtctltglrdasgvtftwtpssgksavqgp
pdrdlcgcysvssvlpcaepwnhgktfictaaypesktpatlsksngtfrpevhllpppseelalnelvtltclargfspkdvlvr
wlqgsqelprekyltwasrqepsqgtttfavtsilrvaaedwkkgdtfscmvghealplafqtidrlagkpthvnsvvmaev
d

40 149. 2H7 scFv IgA WH WCH2 T18CH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc
ccagctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg
taccagcagaagccaggatcctcccccacccctggatttatgcccacccaacctggcttctggagtcctgctcgttcagtgga
45 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac
ccaccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc

tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagtgtcctgcaaggcttctggctacacat
 ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact
 tcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac
 atctgaagactctcggtctatttctgtgcaagagtgtgtactatagtaactcttactggtacttcgatgtctggggcacagggaccac
 5 ggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccac
 ccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag
 agatgcctcaggtgtcacctcactggacgcctcaagtgggaagagcgctgttcaaggaccacctgacctgacctctgtggctg
 ctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgcactgctgcctaccccgagtc
 caagaccccgtaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccggcccgctcgaggag
 10 ctggccctgaacgagctggtgacgtgacgtgacctggcacgtggttcagccccaggatgtgctggttcgctggctgcaggggtc
 acaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccag
 catactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggcccagaggccctgccgctggccttc
 acacagaagaccatcgaccgcttggcgggtaaatgataatctaga

15 Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg
 sgshtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaelvrpgasvkmsckas
 gytfitsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdksstaymqlssltedsavyfcarvvyysnsywy
 fdvwgtgtvtvssdqvpstptpsptpspspcchprlslhrpaledlllgseailtctltglrdasgvtftwtpssgksavqgp
 20 pdrdlcgysvssvlpgaepwnhgktftctaaypesktpltatlsksgntfrpevhllpppseelalnelvtltclargfspkdvivr
 wlqgsqelprekyltwasrqpsqgtttfavtsilrvaadwkkgdtfscmvghelplafitqktidrlagk

150. G19-4 scFv (SSS-P)WH WCH2 WCH3-hCD80TMCT

Nucleotide sequence:

25 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacac
 agactacatctcctgtctgcctctctgggagacagagtcaccatcagttgcagggcaagtcaggacattcgcaattatttaaactgg
 tatcagcagaaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtcctcatcaagggtcagtggcag
 tgggtctggaacagattattctctcaccattgccaacctgcaaccagaagatattgccacttacttttgccaacagggttaatacgttccg
 tggacgttcggaggagcacaaactgtaaccaaaccgggagctcgggtggcgggtggctcgggcgggtggtgggtcgggtggcggg
 30 cggatctatcgatgaggtccagctgcaacagctctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggcctctggttac
 tcattcactggctacatcgtaactggctgaagcagagccatggaaagaaccttgagtggttgacttattaatccatacaaaggctctt
 actacctacaaccagaaattcaagggaaggccacattaactgtagacaagtcacacagcctacatggagctcctcagctctg
 acatctgaagactctgcagcttattactgtgcaagatctgggtactatggtgactcggactggtacttcgatgtctggggcgagggac
 cagggtcacgtctcctctgatctggagcccaaatctctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggg
 35 ggaccgtcagcttctcttcccccaaaacccaaggacaccctcatgatctcccgacccttgaggtcacatgctggtggtggac
 gtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaagccgcgggag
 gagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtga
 aggttccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtaca
 ccctgccccatcccgggatgagctgaccaagaaccaggctcagcctgacctgctgtaaaggcttctatcccagcgacatcgcc
 40 gtggagtgggagagcaatgggcagccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttcttctcta
 cagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgatgaggctctgcacaaccact
 acacgcagaagagcctctcctgtctccgggtaagcggatccttcgaacctgctccatcctgggccattaccttaatctcagtaaat
 ggaattttgtgatgtgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagt
 gtacgccctgtataaatcgat

45

Amino acid sequence:

mdfqvqifsflisasvimsrgvdiqmtqtsslsaslgdrvtiscrasqdirnylnwyqqkpdgtvkllyytsrlhsgvpsrfs
 gsgtdysltianlqpediatyfcqqgntlpwtfgggtklvtkrelgggsgggsgggsgggsidevqlqqsgpelvkpgasmsckas
 gysftgyivnwlkqshgknlewiglinpykglttynqkfkkgkatltvdkssstaymellsitsedsavyycarsgyygsdwyf
 dvwgagttvtvssdlepkssdkthtspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgve
 5 vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltel
 vkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnyhtqkslslspgkads
 nllpswaitlisvngifviccltycfaprerrmerlrresvrpv

151. 2e12 scFv (CCC-P)WH WCH2 WCH3-hCD80TM/CT

10 Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
 atctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaagtta
 atgcagtggtaccaacagaaaccaggacagccaccaaaactcctcatctctgcatccaacgtagaatctggggtccctgccagg
 ttttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattctgtcagcaaagt
 15 aggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtgctcgggagggtgggtcgggt
 ggcgggcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct
 cagggttctcattaaccggctatggtgtaaactgggtcgccagcctccaggaaagggtctggagtggctgggaatgatagggtg
 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtittctaaaaatgaa
 cagctctgaaaactgatgacacagccagataactactgtccagagatggtatagtaacttctactatgttatggactactgggtgcaa
 20 ggaacctcagtcaccgtctcctcagatctggagcccaaatctgtgacaaaactcacatgcccaccgtgccagcacctgaactc
 ctgggggggaccgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtgtgtg
 gtggacgtgagccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgc
 gggaggagcagtacaacagcacgtaccgtgtgtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaa
 gtgcaaggctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt
 25 gtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgaca
 tcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgctcccgtgctggactccgacggctccttctt
 cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcaca
 accactacacgcagaagagcctctccctgtctccgggtaaaaggatccttcgaacctgctccatcctggccattacctaatactca
 gtaaatggaattttgtgatatgctgcctgacctactgcttggcccaagatgcagagagagaaggagggaatgagagattgagaaggg
 30 aaagtgtacgcctgtataaatcgat

Amino acid sequence:

mdfqvqifsflisasvimsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp
 arfsgsgsgtdfslnihpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgggsgvqlkesgpglvapsqslsit
 35 ctvsfsltygvnwvrppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqtdtdaryycardgysnfh
 yyvmdywgqgtsvtvssdlepkscdkthtppcpapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy
 vdgevhnaaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltkn
 qvsltelvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnyhtqkslslsp
 gkadpsnllpswaitlisvngifviccltycfaprerrmerlrresvrpv

40

1D8 scFv (SSS-P)H P238SCH2 WCH3-hCD80TM/CT

Nucleotide sequence:

45 aagcttatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca
 gtctccaacaaccatagctgcatctccaggggagaagggtcacatcacctgccgtgccagctccagtgtaagttacatgtactggtac

cagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagtccaaatcgcttcagtggcagtg
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc
 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcgcgatctc
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctacatgactgtctctgggttctcattaa
 5 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttctaaaaatcaacagctctgcaaactgat
 gacacagccatgtattactgtgccagaatccacttggattactggggccaaggagtcagtggtcacagtctcctctgatctggagccaa
 atcttctgacaaaactcacacaagcccaccgagcccagcacctgaactcctgggggagtcgtcagcttctcctctccccccaaaacc
 aaggacacctcatgatctccggaccttgaggtcacatgcgtgggtggagctgagccacgaagacctgaggtcaagttcaa
 10 ctggtacgtggagggcgtggaggtgcataatgcaagacaaagccgaggagcagtagcaaacagcacgtacctgtggtcag
 cgtcctcaccgtctcgaccaggactgggtgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcg
 agaaaaccatctcaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggtgatgactgaccaaga
 accaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaa
 caactacaagaccacgctcccgtgctggactccgacggctccttctcctctacagcaagctcaccgtggacaagagcaggtggc
 15 agcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggta
 aagcggatccttgaacctgtcccacctgggccattacctaatactcagtaaatggaattttgtgatatgctgcctgacctactgctt
 gccccagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgcctgtataaatcgata

Amino acid sequence:

20 Mdfqvqifsfllisasvimsrgvdivltspttiaaspgekvtitcrasssvsymywyqqksgasplkiwydtsklasgvpnrfsg
 sgshtsyslaintmetedaatyycqwsstpltfsgtkleikrgggsgggsgggsgvqlkeagpglvqptqlslctvsgfs
 ltsdgvhwirpppgklewmgiyydggtdynsaiksrlsirsdtksqvfkinslqtdtdamyyarihfdywgqvmvtv
 ssdlepkssdkthtspspapellggssvflfppkpdltmistrpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree
 25 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsitclvkgyfypsdiav
 ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhlehnhytqkslspsgkadpsnllpswaitlis
 vngifvicltycfaprerrmerlrresvrpv

HCD16lowFL+NL

Nucleotide sequence:

30 aagcttgcgcctatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgaggactgaagatctccaaaggc
 tgtggtgttcttgagcctcaatggtacagggtgctcgagaaggacagtgtagctgaagtgccaggagcctactccctgagga
 caattccacacagtggtttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtcgacgacagt
 ggagagtacaggtgccagacaaacctctccacctcagtgacctgggtgcagctagaagtccatacggctgggtgttgctccaggc
 ccctcgggtgggtgttaaggaggaagacctattcacctgaggtgtcacagctggaagaacactgctctgcataaggtcacatattta
 35 cagaatggcaaggcaggaagtattttcatcataattctgacttctacattccaaaagccacactcaagacagcggctcctacttctgc
 agggggcttgttgggagtaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggttggcagtgtaaccatctcatctc
 ttccacctgggtaccaagctctttctgcttggtgatggtactccttttgcagtggacacaggactatatttctgtgaagacaaacatt
 cgaagctcaacaagagactggaaggaccataaatttaaatggagaaggacctcaagacaaatgacct

40 Amino acid sequence:

mwqlllptallllvsagmrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvddsg
 eyrcqtnlstldpvqlvhighwllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyipkatlkdsqsyf
 crglvgsknsvsetvnititqglavstissffppgyqvsfclvmvllfavdtglyfsvknirsstrdwkdhkfkwrkdpqdk

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended
5 claims.

All patents, patent applications, publications, scientific articles, web sites, and other documents and materials referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced document and material is hereby incorporated by reference to the same extent as if it had
10 been incorporated by reference in its entirety individually or set forth herein in its entirety. Additionally, all claims in this application, and all priority applications, including but not limited to original claims, are hereby incorporated in their entirety into, and form a part of, the written description of the invention. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such
15 patents, applications, publications, scientific articles, web sites, electronically available information, and other referenced materials or documents. Applicants reserve the right to physically incorporate into any part of this document, including any part of the written description, the claims referred to above including but not limited to any original claims.

The specific methods and compositions described herein are representative of
20 preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed
25 herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. Thus, for example, in each instance herein, in embodiments or examples of the present invention, any of the terms “comprising”, “consisting essentially of”, and “consisting of”
30 may be replaced with either of the other two terms in the specification. Also, the terms

“comprising”, “including”, containing”, *etc.* are to be read expansively and without limitation. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. It is also that as used herein and in the appended
5 claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to “a host cell” includes a plurality (for example, a culture or population) of such host cells, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the
10 patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description
15 and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and
20 variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form
25 part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art

will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

CLAIMS

What is claimed is:

1. A non-naturally occurring single chain protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule,
5 said binding domain polypeptide comprising a heavy chain variable region, said heavy chain variable region comprising an amino acid substitution or deletion at one or more amino acid residues; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second
10 polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.
2. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv.
3. A protein of claim 1 wherein the one or more amino acid substitution
15 or deletion in said heavy chain variable region is effective to increase expression or stability of said protein relative to a protein without said deletion or substitution.
4. A protein of claim 1 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.
- 20 5. A protein of claim 4 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.
6. A protein of claim 5 wherein the first target molecule and the second
25 target molecule are different.
7. A protein of claim 5 wherein the first target molecule and the second target molecule are the same.
8. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv comprising one or more amino acid substitution in positions 9, 10, 11, 12,
30 108, 110, 112 in said heavy chain variable region.

9. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv comprising an amino acid substitution at position 11 in said heavy chain variable region.

5 10. A protein of claim 9 wherein the amino acid substituted for the amino acid at position 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

10 11. A protein of claim 9 wherein the amino acid substituted for the amino acid at position 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, and glutamine.

12. A protein of claim 9 where leucine is replaced by serine at position 11.

13. A protein of claim 9 where leucine is replaced by des-leucine at position 11.

15 14. A protein of claim 12 having an increased recombinant expression or stability relative to said protein not having an amino acid substitution at position 11.

15. A protein of claim 14 wherein the expression of said protein having an amino acid substitution at position 11 is 10-100 fold greater than said protein without a substitution at position 11.

20 16. A protein of claim 14 wherein said expression is in mammalian cells.

17. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of said single chain Fv has been deleted.

25 18. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv and said binding domain polypeptide comprises a light chain variable region, wherein said light chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107.

19. A protein of claim 18 wherein the amino acid at position 106 has been substituted or deleted.

20. A protein of claim 2 wherein said binding domain polypeptide binds to a tumor antigen.
21. A protein of claim 2 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.
- 5 22. A protein of claim 2 wherein said binding domain polypeptide binds to a cancer cell antigen.
23. A protein of claim 22 wherein said cancer cell antigen is a surface antigen.
24. A protein of claim 22 wherein said cancer cell antigen is an
10 intracellular antigen.
25. A protein of claim 1 wherein said binding domain polypeptide binds to a B cell antigen.
26. A protein of claim 25 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
- 15 27. A protein of claim 2 wherein said single chain Fv binds to a B cell antigen.
28. A protein of claim 27 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
29. A protein of claim 28 wherein said single chain Fv is selected from
20 the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.
30. A protein of claim 2 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.
- 25 31. A protein of claim 1 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.
32. A protein of claim 31 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.
33. A protein of claim 1 wherein said binding domain polypeptide binds
30 to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10,

CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

5 34. A protein of claim 1 wherein said protein is capable of forming a complex comprising two or more of said proteins.

 35. A protein of claim 34 wherein said complex is a dimer.

 36. A protein of claim 1 wherein said protein is a monomer.

 37. A protein of claim 1 coupled to a drug, toxin, immunomodulator,
10 polypeptide effector, isotope, label, or effector moiety.

 38. A protein of claim 1 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular
15 activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

 39. A protein of claim 38 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other
20 second messengers, ion channels, and secretory pathway components.

 40. A protein of claim 38 which is capable of induction of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

 41. A protein of claim 40 wherein said induction of one or more immune
25 effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

 42. A protein of claim 38 which is capable of cellular activation, wherein said activation leads to changes in cellular transcriptional activity.

 43. A protein of claim 42 wherein said cellular transcriptional activity is
30 increased.

44. A protein of claim 42 wherein said cellular transcriptional activity is decreased.

45. A protein of claim 38 wherein said one or more biologically active molecules is a protease.

5 46. A protein of claim 38 wherein said one or more biologically active molecules is a cytokine.

47. A protein of claim 46 wherein said cytokine is selected from the group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.

10 48. A protein of claim 38 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.

49. A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins.

15 50. A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF.

20 51. A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from the group consisting of saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.

52. A protein of claim 1 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

25 53. A protein of claim 1 wherein said binding domain polypeptide comprises a light chain variable region attached to said heavy chain variable region by a binding domain linker, wherein said binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser.

30 54. A protein of claim 53 comprising three Gly-Gly-Gly-Gly-Ser peptides.

55. A protein of claim 1 wherein said binding domain polypeptide comprises wild type or engineered immunoglobulin variable region obtained from species selected from the group consisting of human, murine, rat, pig, and monkey.

5 56. A protein of claim 1 wherein said binding domain polypeptide comprises a humanized immunoglobulin variable region.

57. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

10 58. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

15 59. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

20 60. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

61. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region.

25 62. A protein of claim 2 wherein said binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region.

63. A protein of claim 1 wherein said connecting region comprises a naturally occurring hinge region selected from the group consisting of a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof,

IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof.

64. A protein of claim 1 wherein said connecting region comprises a human IgE hinge or a portion thereof.

5 65. A protein of claim 1 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

66. A protein of claim 1 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues.

10 67. A protein of claim 1 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

68. A protein of claim 1 wherein said connecting region comprises a glycosylation site.

69. A protein of claim 1 wherein said connecting region has no cysteine residues capable of forming disulfide bonds.

15 70. A protein of claim 1 wherein said connecting region has one cysteine residue.

71. A protein of claim 1 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

20 72. A protein of claim 1 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

73. A protein of claim 1 where said connecting region comprises three cysteine residues and one proline residue, wherein one or more of said cysteine residues is deleted or substituted and said proline residue is substituted or deleted.

25 74. A protein of claim 1 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is substituted or deleted.

75. A protein of claim 74 wherein said wild-type hinge region polypeptide is from human IgG1.

76. A non-naturally occurring single chain protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region comprising one or more amino acid deletion or substitution in positions 9, 10, 11, 12, 108, 110, 112; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

77. A non-naturally occurring single chain Fv protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine is replaced by serine at position 11 in the first framework region of the heavy chain variable region; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

78. A protein of claim 77 further comprising a substitution or deletion of the amino acid at position 10 in the first framework region of the heavy chain variable region.

79. A protein of claim 77 wherein the amino acid substitution at position 11 is effective to increase expression or stability of said single chain Fv protein relative to a single chain Fv protein without said deletion or substitution.

80. A protein of claim 77 wherein the numbering of the residues is the EU index according to Kabat.

81. A protein of claim 77 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine residue is N-

terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

82. A protein of claim 77 wherein said connecting region comprises an IgA hinge region or portion thereof.

5 83. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein said second cysteine residue is replaced by serine and said proline residue is replaced by serine in the connecting region, and said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

10 84. A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, said connecting region comprises a murine IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from murine IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

15 85. A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein said first, second, and third cysteine residues in the connecting region are replaced by serine and said proline residue in the connecting region is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein lysine is replaced by serine at position 322 in said CH2 region.

20 86. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein lysine is replaced by serine at position 322 in said CH2 region.

25 87. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 331 in said CH2 region.

88. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
5 from IgG₁ wherein proline is replaced by serine at position 331 in said CH2 region.

89. A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁
10 wherein threonine is replaced by asparagine at position 256 in said CH2 region.

90. A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprises 2H7 single chain Fv CH2 and CH3 domains
15 from IgG₁ where in the CH2 domain arginine is replaced by glutamine at position 255, threonine is replaced by asparagine at position 256, proline is replaced by alanine at position 257, and glutamic acid is replaced by lysine at position 258.

91. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said
20 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein lysine is replaced by glutamine at position 290 in said CH2 region.

92. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said
25 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein alanine is replaced by proline at position 339 in said CH2 region.

93. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region
30 said first, second, and third cysteine residues are replaced by serine and said proline residue

is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

94. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region
5 said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

95. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region
10 said second cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

96. A protein of claim 81 said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first and second cysteine residues are replaced by serine, said heavy chain constant region
15 comprising CH2 and CH3 domains from IgG₁.

97. A protein of claim 81 said single chain protein comprises a single chain Fv binding domain from a FC2-2 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
20 from IgG₁.

98. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a UCHL-1 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
25 from IgG₁.

99. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 5B9 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises CH2 and CH3
30 domains from IgG₁.

100. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

101. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

102. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

103. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma comprising a connecting region wherein said third cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

104. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first cysteine residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

105. A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said connecting region comprising a murine IgA hinge region, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA and said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

106. A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said connecting region comprising a human IgA hinge region and said heavy chain constant region comprises CH2 and CH3 domains from human IgA, where said CH3 comprises a deletion or substitution in
5 four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

107. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a HD37 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is
10 replaced by serine, and said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

108. A protein of claim 81 where said single chain protein comprises a L6 single chain Fv binding domain, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, and
15 said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

109. A non-naturally occurring single chain Fv protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region where leucine is replaced by serine at position 11 in the first framework region of the heavy chain
20 variable region; wherein said protein has an increased expression or stability in mammalian cells relative to a protein not having said amino acid substitution; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-
25 chain Fv protein is capable of at least one immunological activity.

110. A non-naturally occurring single chain Fv protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine
30 at is replaced by serine at position 11 in the first framework region of the heavy chain variable region; and ii) a second polypeptide comprising an N-terminally truncated IgE

immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

111. A protein of claim 110 wherein the amino acid substitution at position 11 is effective to increase expression or stability of said single chain Fv protein relative to a single chain Fv protein without said deletion or substitution.

112. A protein of claim 110 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma and said heavy chain constant region comprises CH2, CH3, and CH4 domains from IgE.

113. A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma and said heavy chain constant region comprises CH2, CH3, and CH4 domains from murine IgE.

114. A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said heavy chain constant region comprises CH2, CH3, and CH4 domains from murine IgE.

115. A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said heavy chain constant region comprises CH2, CH3, and CH4 domains from human IgE.

116. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein one or both of said second and third cysteine residues is substituted or deleted, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

117. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv.

118. A protein of claim 116 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

119. A protein of claim 118 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

120. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, 112.

121. A protein of claim 117 wherein said binding domain polypeptide is a single chain Fv comprising a light chain variable region, wherein said light chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107.

122. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11.

123. A protein of claim 122 wherein acid at position of 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

124. A protein of claim 122 wherein the amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, and glutamine.

125. A protein of claim 122 wherein leucine is replaced by serine at position 11.

126. A protein of claim 122 wherein leucine is replaced by des-leucine at position 11.

127. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of said single chain Fv has been deleted.

5 128. A protein of claim 121 where leucine is replaced by serine at position 106.

129. A protein of claim 117 wherein said binding domain polypeptide binds to a tumor antigen.

130. A protein of claim 117 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.

10 131. A protein of claim 117 wherein said binding domain polypeptide binds to a cancer cell antigen.

132. A protein of claim 131 wherein said cancer cell antigen is a surface antigen.

15 133. A protein of claim 131 wherein said cancer cell antigen is an intracellular antigen.

134. A protein of claim 116 wherein said binding domain polypeptide binds to a B cell antigen.

135. A protein of claim 135 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

20 136. A protein of claim 117 wherein said single chain Fv binds to a B cell antigen.

137. A protein of claim 136 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

25 138. A protein of claim 137 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.

139. A protein of claim 117 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.

140. A protein of claim 116 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.

141. A protein of claim 136 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.

5 142. A protein of claim 116 wherein said binding domain polypeptide binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand),
10 ICOS, L6, B7-H1, and HLA class II.

143. A protein of claim 116 wherein said protein is capable of forming a complex comprising two or more of said proteins.

144. A protein of claim 143 wherein said complex is a dimer.

145. A protein of claim 116 wherein said protein is a monomer.

15 146. A protein of claim 116 coupled to a drug, toxin, immunomodulator, polypeptide effector, isotope, label, or effector moiety.

147. A protein of claim 116 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active
20 signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

148. A protein of claim 147 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group
25 consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components.

149. A protein of claim 147 which is capable of induction of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

150. A protein of claim 149 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

151. A protein of claim 147 which is capable of cellular activation,
5 wherein said activation leads to changes in cellular transcriptional activity.

152. A protein of claim 151 wherein said cellular transcriptional activity is increased.

153. A protein of claim 151 wherein said cellular transcriptional activity is decreased.

10 154. A protein of claim 147 wherein said one or more biologically active molecules is a protease.

155. A protein of claim 147 wherein said one or more biologically active molecules is a cytokine.

156. A protein of claim 155 wherein said cytokine is selected from the
15 group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.

157. A protein of claim 147 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.

158. A protein of claim 147 A protein which is capable of neutralization of
20 a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins.

159. A protein of claim 147 which is capable of neutralization of a toxin,
wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin,
cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat
25 stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin,
Bordetella pertussis AC toxin, and *Bacillus anthracis* EF.

160. A protein of claim 147 which is capable of neutralization of a toxin,
wherein said toxin is an endotoxin selected from the group consisting of saxitoxins,
tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins,
30 and grayanotoxins.

161. A protein of claim 116 wherein said binding domain polypeptide comprises a light chain variable region attached to said heavy chain variable region by a binding domain linker, wherein said binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser.

5 162. A protein of claim 116 comprising three Gly-Gly-Gly-Gly-Ser peptides.

163. A protein of claim 116 wherein said binding domain polypeptide comprises wild type or engineered immunoglobulin variable region obtained from species selected from the group consisting of human, murine, rat, pig, and monkey.

10 164. A protein of claim 116 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

15 165. A protein of claim 116 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

166. A protein of claim 116 wherein said immunoglobulin heavy chain constant region polypeptide is without a functionally active CH1 domain.

20 167. A protein of claim 116 said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

168. A protein of claim 116 comprising at least a portion of a human constant region.

25 169. A protein of claim 116 wherein said connecting region comprises a naturally occurring hinge region selected from the group consisting of a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof, IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof.

170. A protein of claim 116 wherein said connecting region comprises a human IgE hinge or a portion thereof.

171. A protein of claim 116 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

5 172. A protein of claim 116 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues.

173. A protein of claim 116 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

10 174. A protein of claim 116 wherein said connecting region comprises a glycosylation site.

175. A protein of claim 116 wherein said connecting region has no cysteine residues capable of forming disulfide bonds.

176. A protein of claim 116 wherein said connecting region has one cysteine residue.

15 177. A protein of claim 116 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

178. A protein of claim 116 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

20 179. A protein of claim 116 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

25 180. A protein of claim 179 where said single chain protein comprises a CTLA-4 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 domain.

181. A protein of claim 179 where said single chain protein comprises a CTLA-4 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

5 182. A protein of claim 179 where said single chain protein comprises a FC2-2 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

10 183. A protein of claim 179 where said single chain protein comprises a UCHL-1 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

15 184. A protein of claim 179 where said single chain protein comprises a 5B9 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

20 185. A protein of claim 179 where said single chain protein comprises a 2H7 scFv single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

25 186. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

30 187. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein phenylalanine is replaced by tyrosine at position 405 in said CH3 region.

188. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprises CH2 and CH3 domains from IgG₁ wherein phenylalanine is replaced by alanine at position 405 in said CH3 region.

189. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein tyrosine is replaced by alanine at position 407 in said CH3 region.

190. A protein of claim 189 having an apparent molecular weight of about 75 kDa.

191. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein phenylalanine is replaced by alanine at position 405 and tyrosine is replaced by alanine at position 407 in said CH3 region.

192. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

193. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

194. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first and second cysteine residues are replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

195. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said second cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

5 196. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said third cysteine residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

10 197. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first cysteine residue is replaced by serine, and where said heavy chain constant region comprises 2H7 single chain Fv CH2 and CH3 domains.

15 198. A protein of claim 179 where said single chain protein comprises a HD37 single chain Fv binding domain, wherein in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

199. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG₁.

20 200. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG₂.

25 201. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG₃.

30 202. A protein of claim 179 where said single chain protein comprises a CD-16-6 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region.

203. A protein of claim 179 where said single chain protein comprises a CD-16 receptor binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein
5 proline is replaced by serine at position 238 in said CH2 region.

204. A protein of claim 179 where said single chain protein comprises a 2e12 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁
10 wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

205. A protein of claim 179 where said single chain protein comprises a 10A8 scFv single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is
15 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

20 206. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 40.2.36 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region, and
25 wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

207. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is
30 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains

from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

208. A protein of claim 179 where said single chain protein comprises a
5 single chain Fv binding domain from a G19-4 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and
10 cytoplasmic tail domains.

209. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a G19-4 hybridoma, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

210. A protein of claim 179 where said single chain protein comprises a
15 single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising CD80
20 transmembrane and cytoplasmic tail domains.

211. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
25 from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

212. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said
30 first, second and third cysteine residues are replaced by serine and said proline residue is

replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

213. A protein of claim 179 where said single chain protein comprises a
5 single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

10 214. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said
15 CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

215. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is
20 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

216. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said
25 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

217. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

218. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

219. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

220. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

221. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

222. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
5 from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

223. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 scFv hybridoma, where in the connecting region
10 said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

224. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said
15 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and
20 cytoplasmic tail domains.

225. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
25 from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

226. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is
30 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains

from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

227. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said
5 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

10 228. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mcasp8
15 transmembrane and cytoplasmic tail domains.

229. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
20 from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

230. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said
25 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

231. A protein of claim 179 where said single chain protein comprises a
30 single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said

first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

232. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

233. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

234. A protein of claim 179 where said single chain protein comprises a L6 single chain Fv binding domain, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

235. A protein of claim 179 where said single chain protein comprises a G28-1 single chain Fv comprising a connecting region wherein said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises G28-1 single chain Fv CH2 and CH3 domains.

236. A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein that is less than about 150kD comprising the steps of a) treating the target cell population with a first protein or peptide that binds to cells within said target cell population, and b) treating the target cell

population with a second protein or peptide that capable of at least one of i) binding an Fc receptor, ii) inducing target cell apoptosis, or iii) fix complements, wherein said first protein or peptide molecule is directly connected to said second protein or peptide molecule or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, and wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

237. A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein that is less than about 150kD consisting essentially of the steps of a) treating the target cell population with a first protein or peptide that binds to cells within said target cell population, and b) treating the target cell population with a second protein or peptide that capable of at least one of i) binding an Fc receptor, ii) inducing target cell apoptosis, or iii) fix complements, wherein said first protein or peptide molecule is directly connected to said second protein or peptide molecule or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, and wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

238. A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a light chain variable region comprising one or more amino acid deletion or substitution; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

239. A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a light chain variable region comprising one or more amino acid deletion or substitution in positions 12, 80, 81, 83, 105, 106, and 107; ii) a

second polypeptide comprising a connecting region attached to said first polypeptide; and
iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain
constant region polypeptide attached to the second polypeptide, wherein said non-naturally
occurring single-chain protein is capable of at least one immunological activity.

5 240. A protein of claim 238 wherein the one or more amino acid
substitution or deletion in said light chain variable region is effective to increase expression
or stability of said protein relative to a protein without said deletion or substitution.

 241. A protein of claim 238 wherein said binding domain polypeptide is a
single chain Fv.

10 242. A protein of claim 238 wherein said binding domain polypeptide
comprises an immunoglobulin light chain variable region polypeptide and an
immunoglobulin heavy chain variable region polypeptide.

 243. A protein of claim 242 further comprising a second binding domain
polypeptide capable of binding a second target molecule, said second binding domain
15 polypeptide comprising an immunoglobulin light chain variable region polypeptide and an
immunoglobulin heavy chain variable region polypeptide.

 244. A protein of claim 241 wherein said binding domain polypeptide is a
single chain Fv and said light chain variable region of said single chain Fv has an amino acid
deletion or substitution at position 106.

20 245. A protein of claim 241 wherein said binding domain polypeptide is a
single chain Fv and the amino acid at position 106 of the heavy chain variable region of said
single chain Fv has been deleted.

 246. A protein of claim 238 wherein said binding domain polypeptide is a
single chain Fv and said light chain variable region of said single chain Fv has an amino acid
25 substitution at position 106.

 247. A protein of claim 244 wherein leucine is replaced by serine at
position of 106.

 248. A protein of claim 244 wherein leucine is replaced by des-leucine at
position of 106.

249. A protein of claim 244 wherein the amino acid substituted for the amino acid at position 106 of the single chain Fv light chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.
- 5 250. A protein of claim 244 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11.
251. A protein of claim 241 wherein said binding domain polypeptide binds to a tumor antigen.
- 10 252. A protein of claim 241 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.
253. A protein of claim 241 wherein said binding domain polypeptide binds to a cancer cell antigen.
- 15 254. A protein of claim 253 wherein said cancer cell antigen is a surface antigen.
255. A protein of claim 253 wherein said cancer cell antigen is an intracellular antigen.
256. A protein of claim 238 wherein said binding domain polypeptide binds to a B cell antigen.
- 20 257. A protein of claim 256 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
258. A protein of claim 241 wherein said single chain Fv binds to a B cell antigen.
259. A protein of claim 258 wherein said B cell antigen is selected from
25 the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
260. A protein of claim 259 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.
- 30 261. A protein of claim 238 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.

262. A protein of claim 261 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.

263. A protein of claim 238 wherein said binding domain polypeptide binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8,
5 CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

264. A protein of claim 238 wherein said protein is capable of forming a
10 complex comprising two or more of said proteins.

265. A protein of claim 264 wherein said complex is a dimer.

266. A protein of claim 238 wherein said protein is a monomer.

267. A protein of claim 238 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity,
15 complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

268. A protein of claim 267 which is capable of induction of biologically
20 active signals by activation or inhibition of one or more molecules selected from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components.

269. A protein of claim 267 which is capable of induction of one or more
25 immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

270. A protein of claim 267 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

271. A protein of claim 267 which is capable of cellular activation,
30 wherein said activation leads to changes in cellular transcriptional activity.

272. A protein of claim 271 wherein said cellular transcriptional activity is increased.

273. A protein of claim 271 wherein said cellular transcriptional activity is decreased.

5 274. A protein of claim 267 wherein said one or more biologically active molecules is a protease.

275. A protein of claim 267 wherein said one or more biologically active molecules is a cytokine.

276. A protein of claim 275 wherein said cytokine is selected from the
10 group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.

277. A protein of claim 267 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.

278. A protein of claim 267 which is capable of neutralization of a toxin,
15 wherein said toxin is selected from the group consisting of endotoxins and exotoxins.

279. A protein of claim 267 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin,
20 *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF.

280. A protein of claim 267 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from the group consisting of saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.

25 281. A protein of claim 238 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

282. A protein of claim 238 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region
30 polypeptide.

283. A protein of claim 238 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

5 284. A protein of claim 238 comprising at least a portion of a human constant region.

285. A protein of claim 238 wherein said connecting region comprises a naturally occurring hinge region selected from the group consisting of a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof, IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof.

286. A protein of claim 238 wherein said connecting region comprises a human IgE hinge or a portion thereof.

15 287. A protein of claim 238 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

288. A protein of claim 238 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues.

20 289. A protein of claim 238 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

290. A protein of claim 238 wherein said connecting region comprises a glycosylation site.

291. A protein of claim 238 wherein said connecting region has no cysteine residues capable of forming disulfide bonds.

25 292. A protein of claim 238 wherein said connecting region has one cysteine residue.

293. A protein of claim 238 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

294. A protein of claim 238 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

295. A protein of claim 238 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

296. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, said connecting region comprises at least a portion of an IgA hinge region, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

297. A protein of claim 296 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

298. A protein of claim 297 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

299. A protein of claim 296 wherein said connecting region consists essentially of at least a portion of an IgA hinge region.

300. A protein of claim 296 wherein said connecting region consists essentially of an IgA hinge region.

301. A protein of claim 300 that is expressed as a protein having an apparent molecular weight of at least about 70 kDa.

302. A protein of claim 300 that is expressed as a protein having an apparent molecular weight of between about 70 kDa and about 700 kDa.

303. A protein of claim 296 wherein said connecting region comprises between 10 to 50 amino acids.

304. A protein of claim 296 wherein said connecting region comprises a modified human IgA immunoglobulin hinge region polypeptide.

305. A protein of claim 296 wherein said connecting region has only one cysteine residue.

5 306. A protein of claim 296 wherein said connecting region two cysteine residues.

307. A protein of claim 296 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide in the third polypeptide comprises IgA immunoglobulin heavy chain constant regions.

10 308. A protein of claim 307 comprising CH2 and CH3 domains from IgA immunoglobulin heavy chain constant regions.

309. A protein of claim 308 wherein said IgA CH3 constant region domain comprises a substitution or deletion in one or more amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

15 310. A protein of claim 308 wherein said IgA heavy chain constant region is capable of associating with a J chain polypeptide.

311. A protein of claim 309 wherein said IgA CH3 region domain comprises a deletion in between four and twenty amino acids.

20 312. A protein of claim 309 wherein said IgA region domain comprises a deletion of four amino acids.

313. A protein of claim 309 wherein said IgA region domain comprises a deletion of twenty amino acids.

25 314. A protein of claim 296 wherein said single chain protein comprises a L6 single chain Fv binding domain, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

30 315. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a L6 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

316. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a CTLA-4 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA.

5 317. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a CTLA-4 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA and said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of
10 associating with a J chain polypeptide.

318. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA.

15 319. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, said connecting region comprises a IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in eighteen amino acids that render the IgA heavy chain constant region incapable of associating with a J chain
20 polypeptide.

320. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said connecting region comprises a IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino
25 acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

321. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said connecting region comprises a
30 IgA hinge or portion thereof, said heavy chain constant region comprises CH2 and CH3

domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

5 322. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, said connecting region comprises a IgA hinge or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain
10 polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

 323. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region
15 comprising CH2 and CH3 domains from IgG₁.

 324. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

20 325. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of
25 associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

 326. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region

comprises CH2 and CH3 domains from IgA, wherein the IgA heavy chain constant region is capable of associating with a J chain polypeptide.

327. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a HD37 hybridoma, wherein the connecting region
5 comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA.

328. A non-naturally occurring single chain protein comprising i) a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, and ii) a second polypeptide comprising at least a portion of an IgE
10 immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

329. A protein of claim 328 wherein said binding domain polypeptide comprises a non-IgE variable region.

15 330. A protein of claim 328 wherein said binding domain polypeptide is a single chain Fv selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.

331. A protein of claim 328 wherein said binding domain polypeptide
20 comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

332. A protein of claim 331 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an
25 immunoglobulin heavy chain variable region polypeptide.

333. A protein of claim 332 wherein said immunoglobulin light and heavy chains comprise IgG immunoglobulin light and heavy chains or a portions thereof.

334. A protein of claim 328 wherein said binding domain polypeptide comprises at least a portion of an IgG immunoglobulin variable region.

335. A protein of claim 334 wherein said IgG variable region comprises a heavy chain variable region and a light chain variable region.

336. A protein of claim 328 that is capable of binding to a Fc receptor specific for IgE.

5 337. A protein of claim 328 that does not have IgE variable chain region.

338. A protein of claim 328 wherein the binding domain consist essentially of an IgG immunoglobulin light chain region and a IgG immunoglobulin light and heavy chain region.

10 339. A protein of claim 328 wherein said IgE immunoglobulin heavy chain constant region polypeptide comprises CH2 and CH3 domains.

340. A protein of claim 328 wherein said IgE immunoglobulin heavy chain constant region polypeptide comprises CH2, CH3 and CH4 domains.

15 341. A protein of claim 328 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11.

342. A protein of claim 328 wherein said single chain protein is a single chain Fv selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.

20 343. A protein of claim 328 wherein said single chain protein is a single chain Fv comprising a 2H7 single chain Fv or a G28-1 single chain Fv.

25 344. A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a non-naturally occurring single chain protein, said protein comprising a first polypeptide comprising a binding domain polypeptide, said binding domain polypeptide comprising at least a portion of an IgG variable region and being capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an IgE immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

345. The method of claim 344 wherein said protein is administered into the blood stream of a patient.

346. The method of claim 344 wherein monocytes are activated but mast cells are not activated as a result of administration of the protein.

5 347. The method of claim 344 wherein the protein is administered or coadministered with a histamine release blocker.

348. A method of depleting cells in an animal comprising administering a modified IgE protein into the blood stream of an animal.

10 349. The method of claim 348 wherein the modified IgE protein is administered or coadministered with a histamine release blocker.

350. A protein of claim 328 wherein said connecting region comprises a IgG hinge or portion thereof, and said heavy chain constant region is from IgE and comprises CH3 and CH4 domains without a CH2 domain.

15 351. A protein of claim 328 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12, and said heavy chain constant region comprises IgE CH2, CH3, and CH4 domains, and wherein said heavy chain constant region is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

20 352. A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; ii) a second polypeptide comprising a connecting region attached to said first polypeptide, said connecting region comprising three cysteine residues and one proline residue, wherein one or more of said cysteine residues is deleted or substituted; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-
25 chain protein is capable of at least one immunological activity.

353. A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is not substituted or deleted, and where said second and third
30 cysteine are substituted or deleted.

354. A protein of claim 353 wherein said second and third cysteine residues are substituted by another amino acid.

355. A protein of claim 354 wherein said second and third cysteine residues are substituted by serine residues.

5 356. A protein of claim 352 that is expressed as a homogeneous protein having an apparent molecular of about 75 kDa.

357. A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein
10 said first cysteine residue is substituted or deleted.

358. A protein of claim 352 where said second and third cysteine residues in said connecting region are not substituted or deleted.

359. A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein
15 said second cysteine residue is substituted or deleted.

360. A protein of claim 352 wherein said first and third cysteine residues in said connecting region are not substituted or deleted.

361. A protein of claim 352 wherein said connecting region comprises
20 first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said third cysteine residue is substituted or deleted.

362. The protein of claim 10 where said first and second cysteine residues in said connecting region are not substituted or deleted.

25 363. A protein of claim 352 wherein said connecting region comprises between 5 and 65 amino acids.

364. A protein of claim 352 wherein said connecting region comprises between 10 and 50 amino acids.

365. A protein of claim 352 wherein said connecting region comprises
30 between 15 and 35 amino acids.

366. A protein of claim 352 wherein said connecting region comprises at least five consecutive hinge region amino acids.

367. A protein of claim 352 wherein said connecting region comprises at least ten consecutive hinge region amino acids.

5 368. A protein of claim 352 wherein said connecting region comprises at least fifteen consecutive hinge region amino acids.

369. A protein of claim 353 wherein said second and third cysteines are replaced by serine residues and said connecting region comprises at least a portion of a IgG₁ hinge region.

10 370. A protein of claim 369 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG₁ hinge region.

371. A protein of claim 353 wherein said second and third cysteines are replaced by serine residues and said connecting region comprises at least a portion of a IgG₂ hinge region.

15 372. A protein of claim 371 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG₂ hinge region.

373. A protein of claim 353 wherein said second and third cysteine residues are replaced by serine residues and said connecting region comprises at least a portion of a IgG₃ hinge region.

20 374. A protein of claim 373 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG₃ hinge region.

375. A protein of claim 353 wherein said second and third cysteine residues are replaced by serine residues and said connecting region comprises at least a portion of a IgG₄ hinge region.

25 376. A protein of claim 375 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG₄ hinge region.

377. A protein of claim 352 wherein said second and third cysteine residues are substituted or deleted and said proline residue is substituted or deleted, wherein said heavy chain constant region polypeptide comprises a CH3 domain wherein tyrosine is

replaced by alanine at position 407, and wherein said protein has an apparent molecular weight of about 75kDa.

378. A protein of claim 352 wherein said first and third cysteine residues are substituted or deleted and said proline residue is substituted or deleted, wherein said heavy chain constant region polypeptide comprises a CH3 domain wherein tyrosine is replaced by alanine at position 407, and wherein said protein has an apparent molecular weight of about 75kDa.

379. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein one or both of said second and third cysteine residues is substituted or deleted, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

380. A protein of claim 352 wherein said connecting region comprises a modified human IgG1 immunoglobulin hinge region polypeptide.

381. A protein of claim 352 wherein said connecting region has only one cysteine residue.

382. A protein of claim 381 wherein said connecting region comprises a modified human IgG1 immunoglobulin hinge region polypeptide.

383. A protein of claim 352 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

384. A protein of claim 352 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2

constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

385. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide, said CH3 constant region polypeptide comprising one or more amino acid substitution or deletion that inhibits two of said proteins from associating together to form a non covalent dimer, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

386. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide, said CH3 constant region polypeptide comprising one or more amino acid substitution or deletion at positions 405 and 407, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

387. A protein of claim 252 or 253 comprising amino acid substitutions at position 405 and position 407.

388. A protein of claim 387 wherein phenylalanine is replaced with alanine at position 405 and tyrosine is substituted with alanine at position 407.

389. A protein of claim 387 having an apparent molecular weight of between about 40 kDa and about 50 kDa.

390. A protein of claim 387 that has a biological activity of an agonist or antagonist.

391. A protein of claim 390 that has a biological activity of an antagonist.

392. A protein of claim 387 wherein only one amino acid at positions 405 and 407 is substituted.

393. A protein of claim 392 having an apparent molecular weight of between about 70 kDa and about 80 kDa.
394. A protein of claim 387 where phenylalanine is replaced by alanine at position 405.
- 5 395. A protein of claim 387 where phenylalanine is replaced by tyrosine at position 405.
396. A protein of claim 386 where tyrosine is replaced by alanine at position 407.
- 10 397. A protein of claim 386 comprising amino acid substitutions at position 405 and position 407.
398. A protein of claim 393 wherein phenylalanine is replaced by alanine at position 405 and tyrosine is replaced by alanine at position 407.
399. A polynucleotide that encodes a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109.
- 15 400. A polynucleotide that encodes a protein according to any one of claims 116, 120, 121, and 180-235.
401. A polynucleotide that encodes a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386.
- 20 402. A polynucleotide of any one of claims 399-401 that is operably linked to a promoter or other sequence that enhances expression of the polynucleotide in a cell.
403. A cell containing a polynucleotide of any one of claims 399-401.
404. A recombinant vector capable of expressing a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109.
- 25 405. A recombinant vector capable of expressing a protein according to any one of claims 116, 120, 121, and 180-235.
406. A method of expressing a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109 under conditions in which the protein is expressed.
407. A method of expressing a protein according to any one of claims 116, 120, 121, and 180-235 under conditions in which the protein is expressed.

408. A method of expressing a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386 under conditions in which the protein is expressed.

409. A composition comprising a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386 in combination with one or more additional
5 therapeutic compounds.

410. A method of treating a subject comprising the steps of administering to said subject a one or more first compound according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386, administering or coadministering a second compound to the subject in combination said first compound, wherein the treatment is effective to treat or
10 prevent a disease, ailment, or undesired condition.

411. The method according to claim 410 wherein the second compound is selected from the group consisting of a chemotherapeutic compound, therapeutic drug, angiogenesis inhibitors, steroids, B-cell activators, T-cell activators, colony stimulating factors, tumor necrosis factors, interferons, antibody, binding construct of the invention,
15 gene therapy, retinoids, surgical procedures, alkylating agents, nucleoside analogues, topoisomerase II, VEGF, IFN-alpha, DMAR agents, interleukin-1, glucocorticoids, p38 inhibitors, interleukin-4, interleukin-6, interleukin-2, interleukin-12, IFN-γ, GM-CSF, G-CSF, M-CSF, anti-CTLA-4, Bcl-2 antisense, vitamin A, anti-CD19, anti-CD20, anti-CD22, anti-CD28 or anti-CD3.

20

412. A method for displaying recombinant molecules, which molecules include a native or engineered immunoglobulin heavy chain variable region, the improvement comprising a immunoglobulin heavy chain region that includes one or more mutation, substitution, alteration, and/or deletion at one or more amino acid residue
25 corresponding positions 9, 10, 11, 12, 108, 110, and 112 in said heavy chain variable region.

413. A non-naturally occurring single chain antigen-binding protein comprising protein having a formula selected from the group consisting of 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3, 2H7 scFv VH L11S IgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgAH WIgACH2 T4CH3, 2H7 scFv VH
30 L11S (SSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3,

2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3, 2HU scFv VH L11S (CSS-S) H P331S
 CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3, 2H7 scFv VH L11S
 (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H K290Q
 CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3, G28-1 scFv (SSS-S) H
 5 WCH2 WCH3, G28-1 scFv IgAH WCH2 WCH3, G28-1 scFv VH L11S (SSS-S) H WCH2
 WCH3, G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (CSC-S) H
 WCH2 WCH3, G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3, CTLA4 (SSS-S) H
 P238SCH2 WCH32, CTLA4 (CCC-P) WH WCH2 WCH3, FC2-2 scFv (SSS-S) H WCH2
 WCH3, FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3, UCHL-1 scFv (SSS-S) H WCH2
 10 WCH3, UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3, 5B9 scFv (SSS-S) H WCH2
 WCH3, 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 WCH3,
 2H7 scFv (SSS-S) H P238SCH2 WCH3, 2H7 scFv IgAH WCH2 WCH3, 2H7 scFv IgAH
 WIgACH2 T4CH3, 2H7 scFv IgAH WIgACH2 WCH3 + JChain, 2H7 scFv (CCC-P) WH
 WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 F405YCH3, 2H7 scFv (SSS-S) H WCH2
 15 F405ACH3, 2H7 scFv (SSS-S) H WCH2 Y407ACH3, 2H4 scFv (SSS-S) HWCH2 F405A,
 Y407ACH3, 2H7 scFv (CSS-S) H WCH2 WCH3, 2H7 scFv (SCS-S) H WCH2 WCH3,
 2H7 scFv (SSC-P) H WCH2 WCH3, 2H7 scFv (CSC-S) H WCH2 WCH3, 2H7 scFv (CCS-
 P) H WCH2 WCH3, 2H7 scFv (SCC-P) H WCH2 WCH3, 2H7 scFv VH L11S (SSS-S) H
 WCH2 WCH3, 2H7 scFv VH L11S (CSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (SCS-
 20 S) H WCH2 WCH3, G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3, G28-1 scFv VH
 L11S (SCC-P) H WCH2 WCH3, G28-1 scFv VH L11S mIgE CH2 CH3 CH4, G28-1 scFv
 VH L11S mIgAH WIgACH2 T4CH3, G28-1 scFv VH L11S hIgE CH2 CH3 CH4, G28-1
 scFv VH L11S hIgAH WIgACH2 T4CH3, HD37 scFv IgAH WCH2 WCH3, HD37 scFv
 (SSS-S) H WCH2 WCH3, HD37 scFv VH L11S (SSS-S) H WCH2 WCH3, L6 scFv IgAH
 25 WCH2 WCH3, L6 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv-llama IgG1, 2H7
 scFv-llama IgG2, 2H7 scFv-llama IgG3, CD16-6 low (ED)(SSS-S) H P238SCH2 WCH3,
 CD16-9 high (ED)(SSS-S) H P238SCH2 WCH3, 2e12 scFv (SSS-s)H P238SCH2 WCH3—
 hCD80TM/CT, 10A8 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 40.2.36 scFv
 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 2H7 scFv (SSS-s)H P238SCH2 WCH3—
 30 hCD80TM/CT, G19-4 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 2e12 scFv (SSS-

s)H WCH2 WCH3—hCD80TM/CT, 2e12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT,
 2e12 scFv IgE CH2CH3CH4—hCD80TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—
 mFADD-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—mFADD-TM/CT, 2e12 scFv (SSS-
 s)H WCH2 WCH3—mcasp3-TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp3-
 5 TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp8--TM/CT, 2e12 scFv (SSS-s)H
 P238SCH2 WCH3—mcasp8-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—hcasp3--
 TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp3-TM/CT, 2e12 scFv (SSS-s)H
 WCH2 WCH3—hcasp8--TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp8-
 TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 1D8 scFv--hIgG1
 10 (SSS-s)H WCH2 WCH3—hCD80TM/CT, 1D8 scFv—mIgAT4—hCD80TM/CT, 1D8
 scFv--hIgE—hCD80TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mFADD-
 TM/CT, 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mFADD-TM/CT, 1D8 scFv--hIgG1
 (SSS-s)H WCH2 WCH3—mcasp3-TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2
 WCH3—mcasp3-TM/CT, 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasp8--TM/CT,
 15 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT, 1D8 scFv--hIgG1 (SSS-
 s)H WCH2 WCH3—hcasp3--TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—
 hcasp3-TM/CT, 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcasp8--TM/CT, 1D8 scFv--
 hIgG1 (SSS-s)H P238SCH2 WCH3—hcasp8-TM/CT, L6 scFv (SSS-S) H WCH2 WCH3,
 2H7 scFv CD154 (L2), 2H7 scFv CD154 (S4), CTLA4 IgAH IGACH2CH3, CTLA4 IgAH
 20 IgACH2 T4CH3, 2H7 scFv IgAH IgACH2CH3, 2H7 scFv IgAH IgAHCH2 T18CH3, 2H&-
 40.2.220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-ccd20-anti-cd40), 2H7 scFv IgAH
 IgACH2 T4CH3-hCD89 TM/CT, G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT,
 and 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT.

25

30

ABSTRACT

The invention relates to novel binding domain-immunoglobulin fusion proteins that feature a binding domain for a cognate structure such as an antigen, a counterreceptor or the like, a wild-type IgG1, IGA or IgE hinge-acting region, *i.e.*, IgE CH2, region polypeptide or a mutant IgG1 hinge region polypeptide having either zero, one or two cysteine residues, and immunoglobulin CH2 and CH3 domains, and that are capable of ADCC and/or CDC while occurring predominantly as polypeptides that are compromised in their ability to form disulfide-linked multimers. The fusion proteins can be recombinantly produced at high expression levels. Also provided are related compositions and methods, including cell surface forms of the fusion proteins and immunotherapeutic applications of the fusion proteins and of polynucleotides encoding such fusion proteins.

C:\301916_2.DOC

Fig. 1

2H7scFv-Ig cDNA and predicted amino acid sequence:

	HindIII	NcoI	2H7 V _L Leader Peptide→	
	~~~~~	~~~~~		
			M D F Q V Q I F S F L L I S A S	
1	AAGCTTGCCG	CCATGGATTT	TCAAGTGCAG	ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA
			2H7 V _L →	
			V I I A R G Q I V L S Q S P A I L S A S	
61	GTCATAATTG	CCAGAGGACA	AATTGTTCTC	TCCCAGTCTC CAGCAATCCT GTCTGCATCT
			P G E K V T M T C R A S S S V S Y M H W	
121	CCAGGGGAGA	AGGTCACAAT	GACTTGCAGG	GCCAGCTCAA GTGTAAGTTA CATGCACTGG
	BamHI			
	~~~~~			
	Y Q Q K P G S S P K P W I Y A P S N L A			
181	TACCAGCAGA	AGCCAGGATC	CTCCCCAAA	CCCTGGATTT ATGCCCCATC CAACCTGGCT
	S G V P A R F S G S G S G T S Y S L T I			
241	TCTGGAGTCC	CTGCTCGCTT	CAGTGGCAGT	GGGTCTGGGA CCTCTTACTC TCTCACAATC
	S R V E A E D A A T Y Y C Q Q W S F N P			
301	AGCAGAGTGG	AGGCTGAAGA	TGCTGCCACT	TATTACTGCC AGCAGTGGAG TTTTAACCCA
	(Gly ₄ Ser) ₃ Linker			
	P T F G A G T K L E L K G G G G S G G G			
361	CCCACGTTCG	GTGCTGGGAC	CAAGCTGGAG	CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT
			2H7 V _H →	
	G S G G G G S S Q A Y L Q Q S G A E L V			
421	GGATCTGGAG	GAGGTGGGAG	CTCTCAGGCT	TATCTACAGC AGTCTGGGGC TGAGCTGGTG
	R P G A S V K M S C K A S G Y T F T S Y			
481	AGGCCTGGGG	CCTCAGTGAA	GATGTCCTGC	AAGGCTTCTG GCTACACATT TACCAGTTAC
	N M H W V K Q T P R Q G L E W I G A I Y			
541	AATATGCACT	GGGTAAAGCA	GACACCTAGA	CAGGGCCTGG AATGGATTGG AGCTATTTAT
	P G N G D T S Y N Q K F K G K A T L T V			
601	CCAGGAAATG	GTGATACTTC	CTACAATCAG	AAGTTCAAGG GCAAGGCCAC ACTGACTGTA
	D K S S S T A Y M Q L S S L T S E D S A			
661	GACAAATCCT	CCAGCACAGC	CTACATGCAG	CTCAGCAGCC TGACATCTGA AGACTCTGCG
	V Y F C A R V V Y Y S N S Y W Y F D V W			
721	GTCTATTTCT	GTGCAAGAGT	GGTGACTAT	AGTAACTCTT ACTGGTACTT CGATGTCTGG

Fig. 1 (continued)

BclI
~~~~~human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H  
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CAGGAGCCCA AATCTTGTGA CAAAACCTCAC

841 T C P P C P A P E L L G G P S V F L F P  
ACATGCCCAC CGTGCCCAGC ACCTGAACTC CTGGGGGGAC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V  
CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG

961 D V S H E D P E V K F N W Y V D G V E V  
GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S  
CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S  
GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R  
AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S  
GAACCACAGG TGTACACCCT GCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

1261 L T C L V K G F Y P S D I A V E W E S N  
CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F  
GGGCAGCCGG AGAACAACCTA CAAGACCACG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC

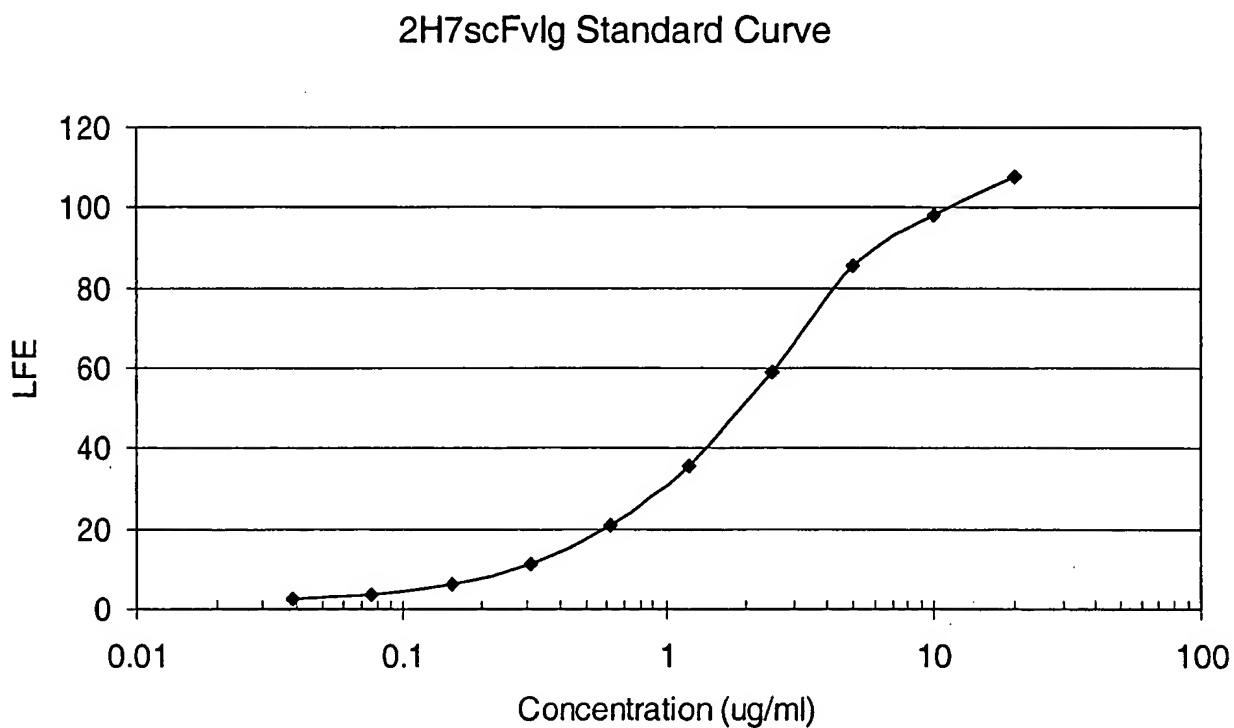
1381 F L Y S K L T V D K S R W Q Q G N V F S  
TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

1441 C S V M H E A L H N H Y T Q K S L S L S  
TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

XbaI  
~~~~~

1501 P G K * S R
CCGGGTAAAT GATCTAGA

Fig. 2
Production Levels of 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3
by Stable CHO Lines



Clone	LFE @ 1:50 Estimated Concentration ($\mu\text{g/ml}$)
D2	26.156
IIIC6	25.755
IVA3	28.661
Spent bulk	29.664

Fig. 3
SDS-PAGE Analysis of
2H7 scFvIgG1 (SSS-S)H WCH2 WCH3 Protein.

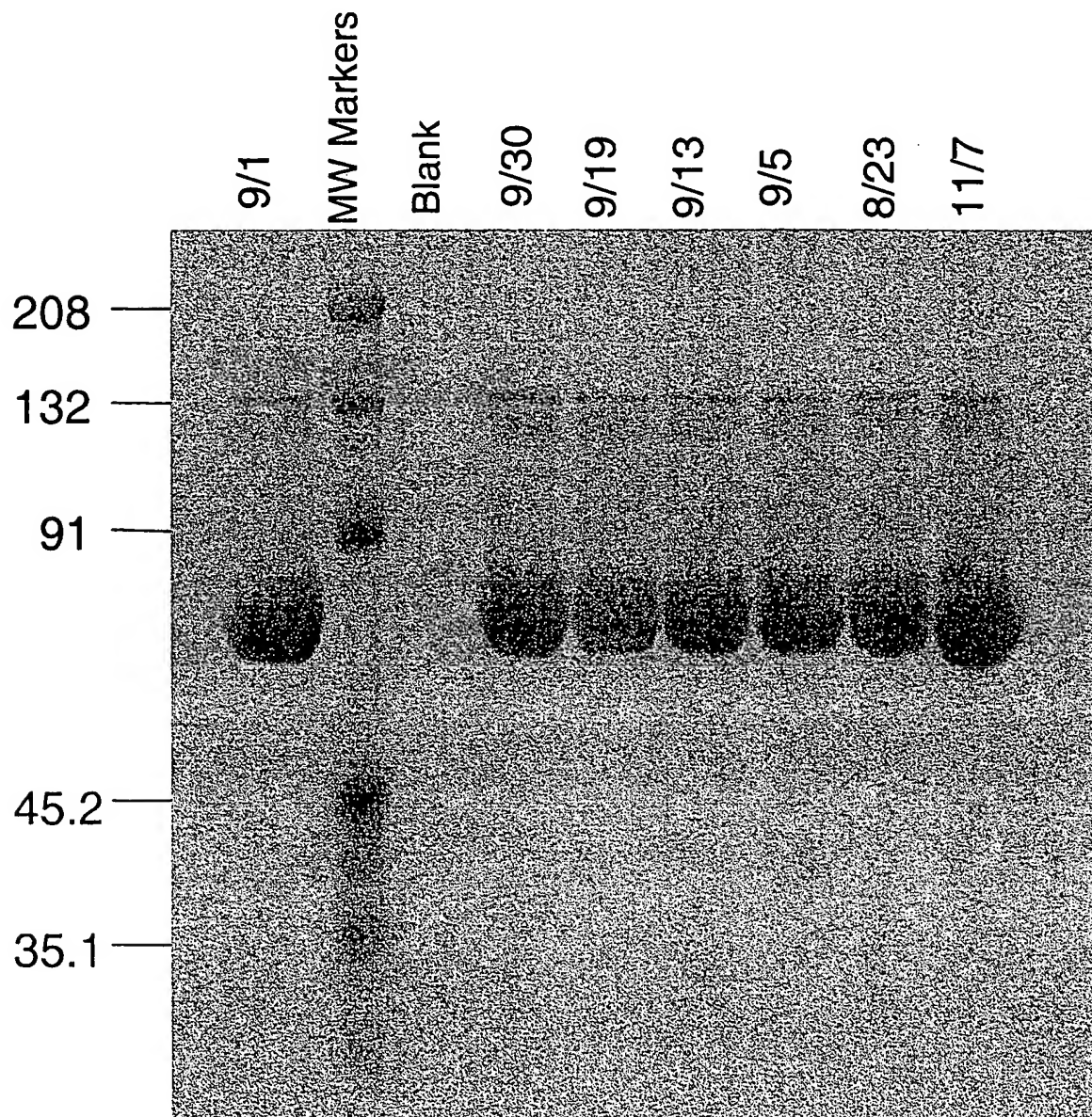


Fig. 4A

Complement Mediated B Cell Killing After Binding of
CD20-targeted 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3:

2H7scFv-Ig Concentration	RAMOS # live cells/total cells	BJAB # live cells/total cells
20 µg/ml + complement	- 0.16	- 0.07
5 µg/ml + complement	- 0.2	- N.D.
1.25 µg/ml + complement	- 0.32	- 0.1
Complement alone	- 0.98	- 0.94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

Fig. 4B

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-IgG1
(SSS-S)H WCH2 WCH3:

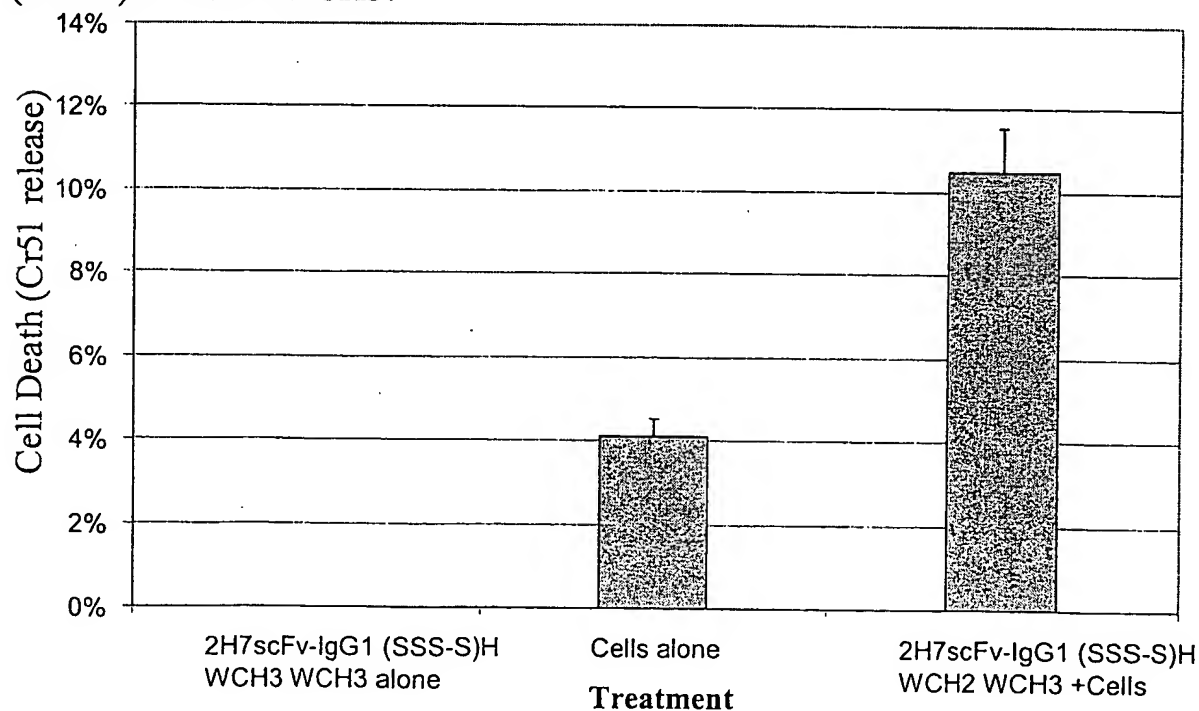


Fig. 5

Effects of Crosslinking of CD20 and CD40 Cell Surface Receptors
on B Cell Proliferation:

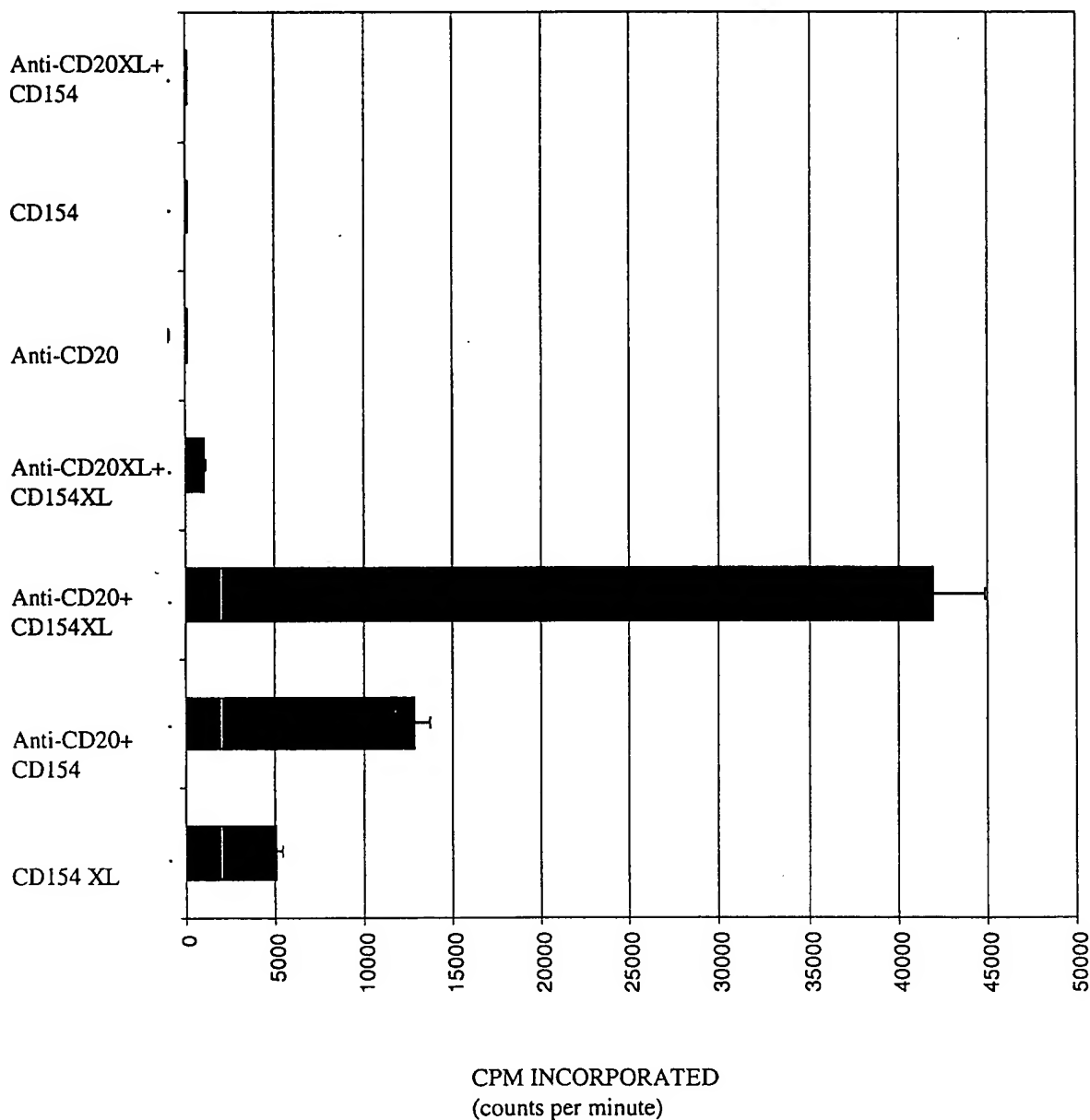


Fig. 6

Effect of Simultaneous ligation of CD20 and CD40
on CD95 and apoptosis.

Fig. 6A.

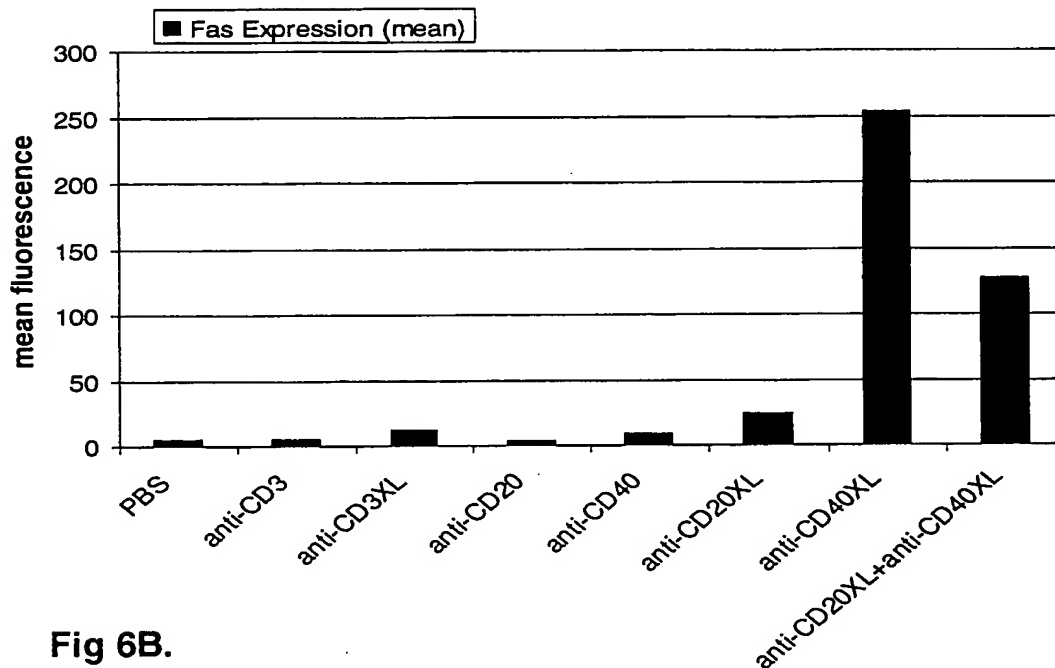


Fig 6B.

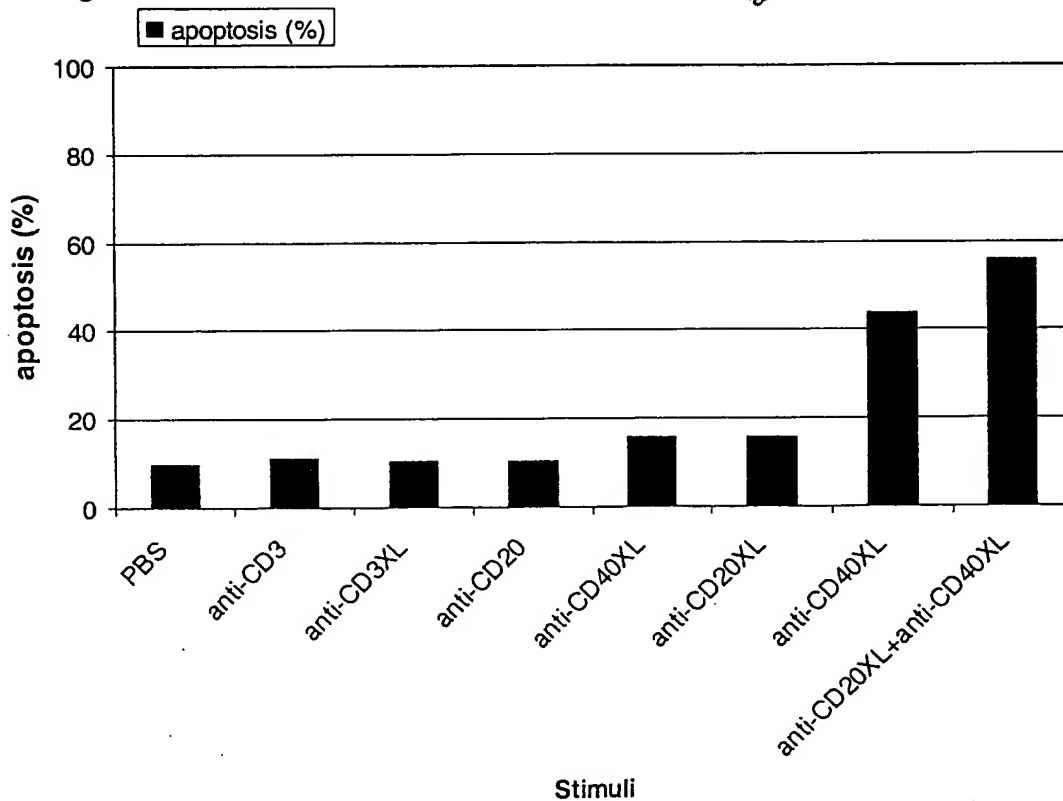


Fig. 7A (continued)

human CD154/amino acid 48→

```

                                     Bcl/Bam hybrid site
      G T G T T V T V S D P R R L D K I E D E
781  GGCACAGGGA CCACGGTCAC CGTCTCTTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA

      R N L H E D F V F M K T I Q R C N T G E
841  AGGAATCTTC ATGAAGATTT TGTATTTCATG AAAACGATAC AGAGATGCAA CACAGGAGAA

      R S L S L L N C E E I K S Q F E G F V K
901  AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

                                     BclI
      D I M L N K E E T K K E N S F E M Q K G
961  GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT

      BclI
      -----
      D Q N P Q I A A H V I S E A S S K T T S
1021 GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACATCT

      V L Q W A E K G Y Y T M S N N L V T L E
1081 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCCTGGAA

      N G K Q L T V K R Q G L Y Y I Y A Q V T
1141 AATGGGAAAC AGCTGACCGT TAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

                                     HindIII
      -----
      F C S N R E A S S Q A P F I A S L C L K
1201 TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

      S P G R F E R I L L R A A N T H S S A K
1261 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

      P C G Q Q S I H L G G V F E L Q P G A S
1321 CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

                                     NcoI
      -----
      V F V N V T D P S Q V S H G T G F T S F
1381 GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

      XhoI          XbaI
      -----
      G L L K L E * * S R
1441 GGCTTACTCA AACTCGAGTG ATAATCTAGA
```

BINDING CONSTRUCTS &
METHODS OF USE THEREOF

Ledbetter, Jeffrey A.

Docket: 49076.000004.CIP2

Fig. 7B.

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

```
HindIII      NcoI
-----
2H7 VL Leader Peptide→
      M D F Q V Q I F S F L L I S A S
1  AAGCTTGCCG CC  ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                2H7 VL →
      V I I A R G Q I V L S Q S P A I L S A S
61  GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

      P G E K V T M T C R A S S S V S Y M H W
121  CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                                BamHI
                                -----
      Y Q Q K P G S S P K P W I Y A P S N L A
181  TACCAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

      S G V P A R F S G S G S G T S Y S L T I
241  TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

      S R V E A E D A A T Y Y C Q Q W S F N P
301  AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly4Ser)3 Linker →
      P T F G A G T K L E L K G G G G S G G G
361  CCCACGTTTC GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                2H7 VH →
      G S G G G G S S Q A Y L Q Q S G A E L V
421  GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R P G A S V K M S C K A S G Y T F T S Y
481  AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

      N M H W V K Q T P R Q G L E W I G A I Y
541  AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

      P G N G D T S Y N Q K F K G K A T L T V
601  CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

      D K S S S T A Y M Q L S S L T S E D S A
661  GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

      V Y F C A R V V Y Y S N S Y W Y F D V W
721  GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
```

Fig. 7B

human CD154/amino acid 108 →

BclI/Bam hybrid site

BclI
G T G T T V T V S D P E N S F E M Q K G
781 GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI

D Q N P Q I A A H V I S E A S S K T T S
841 GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACATCT

V L Q W A E K G Y Y T M S N N L V T L E
901 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCTTGAA

N G K Q L T V K R Q G L Y Y I Y A Q V T
961 AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

F C S N R E A S S Q A P F I A S L C L K
1021 TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

S P G R F E R I L L R A A N T H S S A K
1081 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

P C G Q Q S I H L G G V F E L Q P G A S
1141 CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI

V F V N V T D P S Q V S H G T G F T S F
1201 GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XhoI XbaI

G L L K L E * * S R
1261 GGCTTACTCA AACTCGAGTG ATAATCTAGA

Fig. 8

Simultaneous Binding of 2H7scFv-CD154
Fusion Proteins to CD20 and CD40

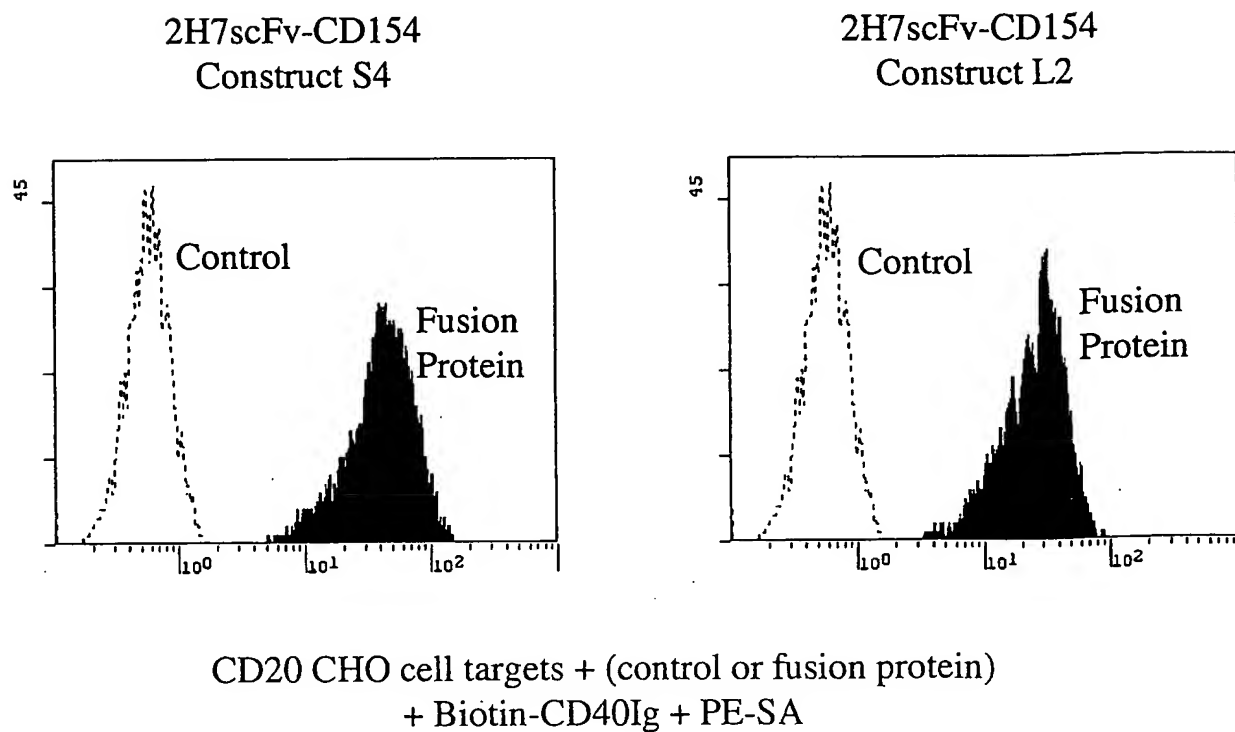
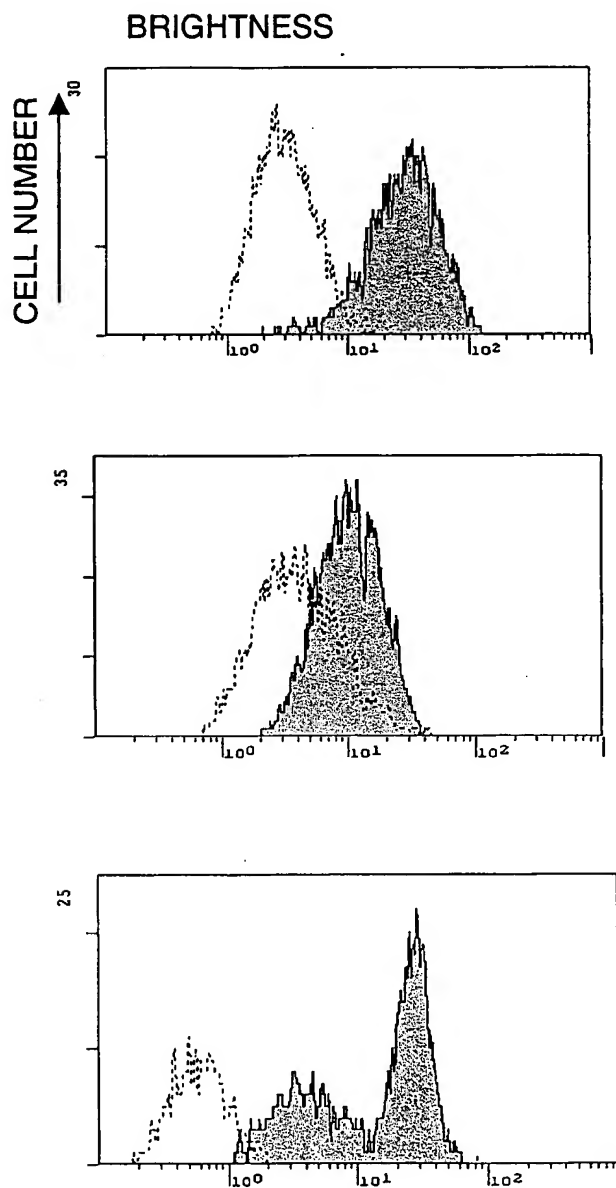


Fig. 9

Induction of Apoptosis Measured by Binding of Annexin V after
incubation with 2H7scFv-CD154



.....control supernatant ____2H7scFv-CD154 supernatant

Fig. 10

Proliferation of T51 B Cell Line After Incubation with 2H7
scFv-CD154 S4 or 2H7 scFv-CD154 L2 Constructs

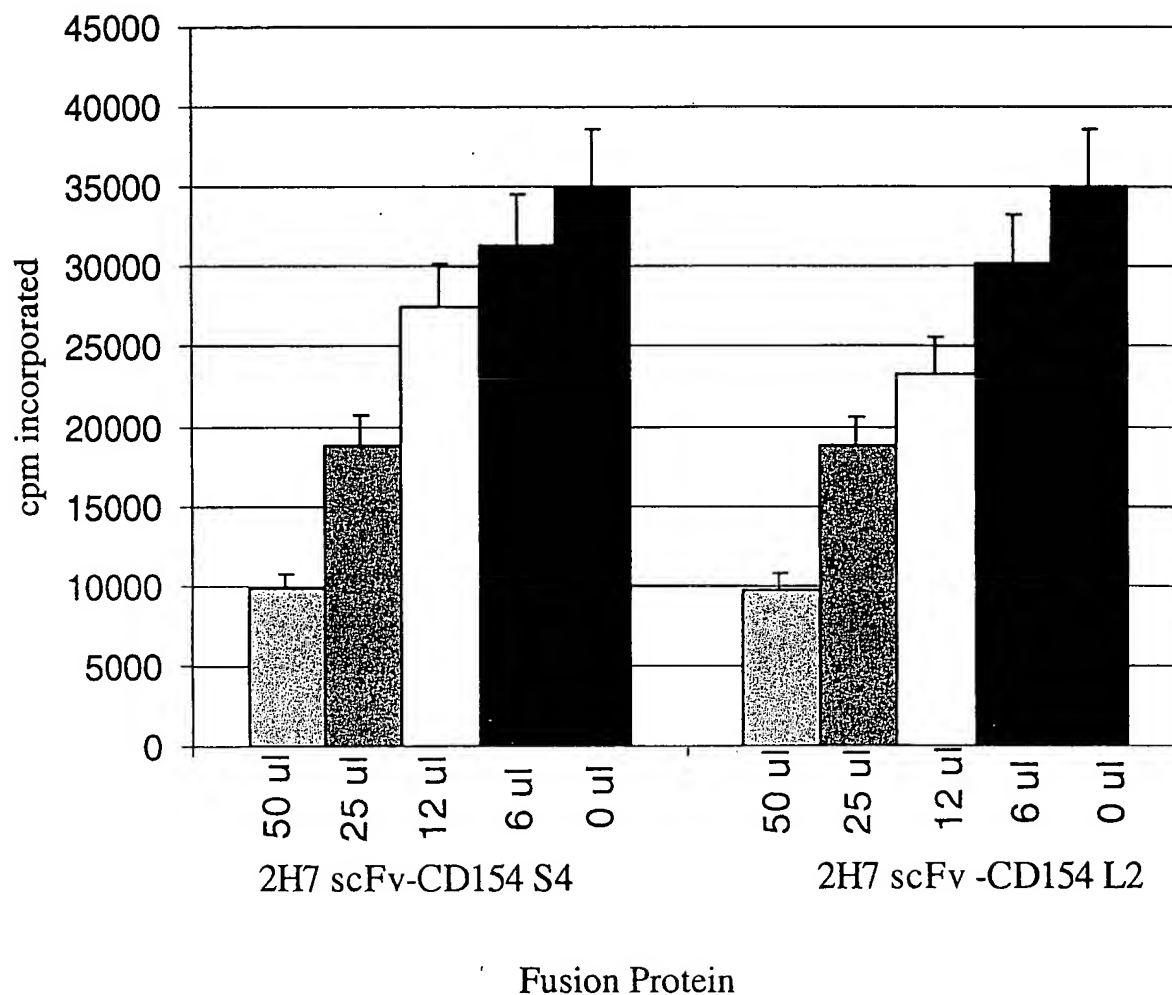


Fig. 11

Schematic Representation of 2H7 scFvIg Constructs

2H7 scFvIgG (SSS-S)H WCH2 WCH3

OR 2H7 scFvIgG1 (SSS-S)H P238SCH2 WCH3 : 2H7 scFv

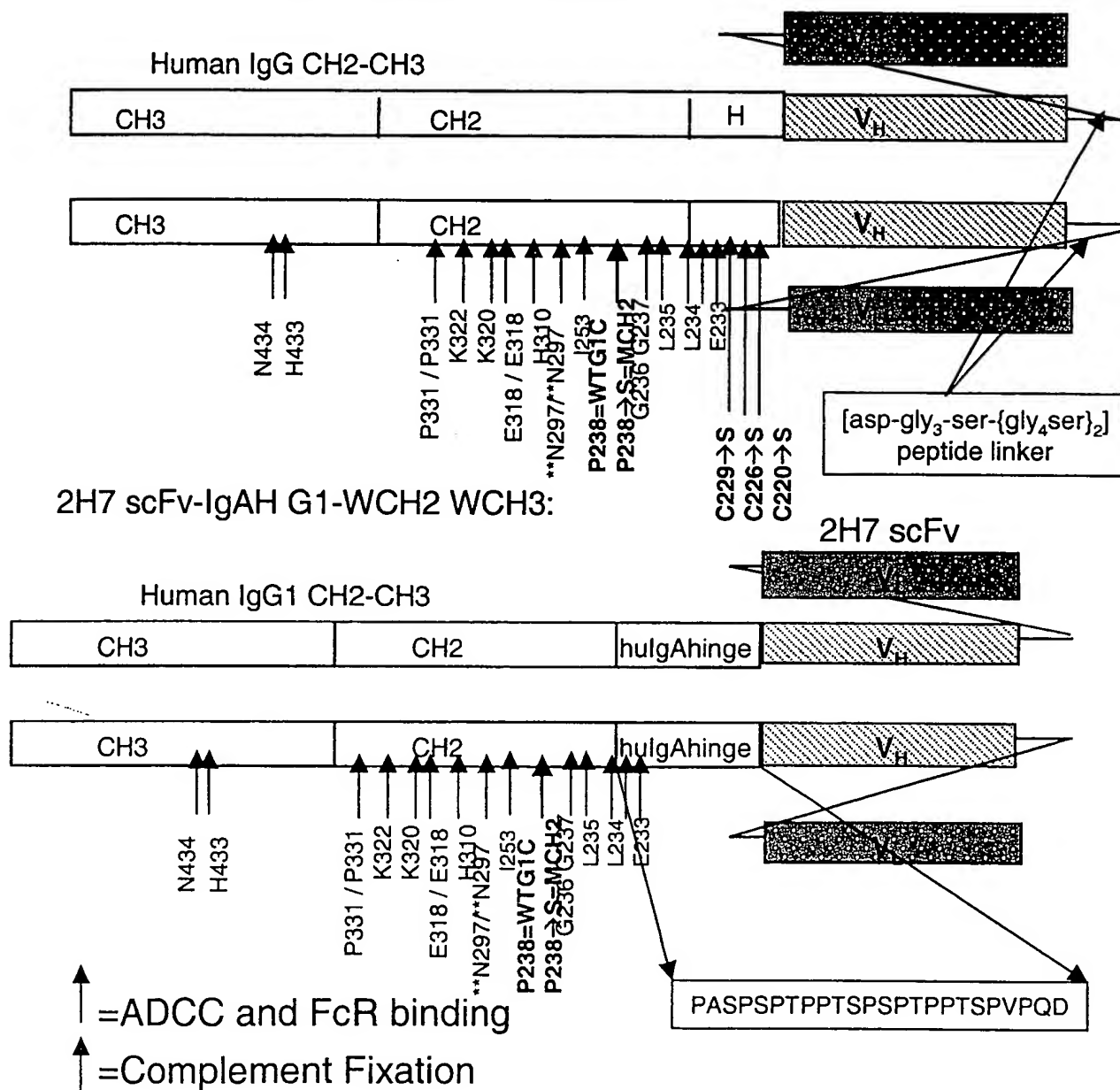


Fig. 12

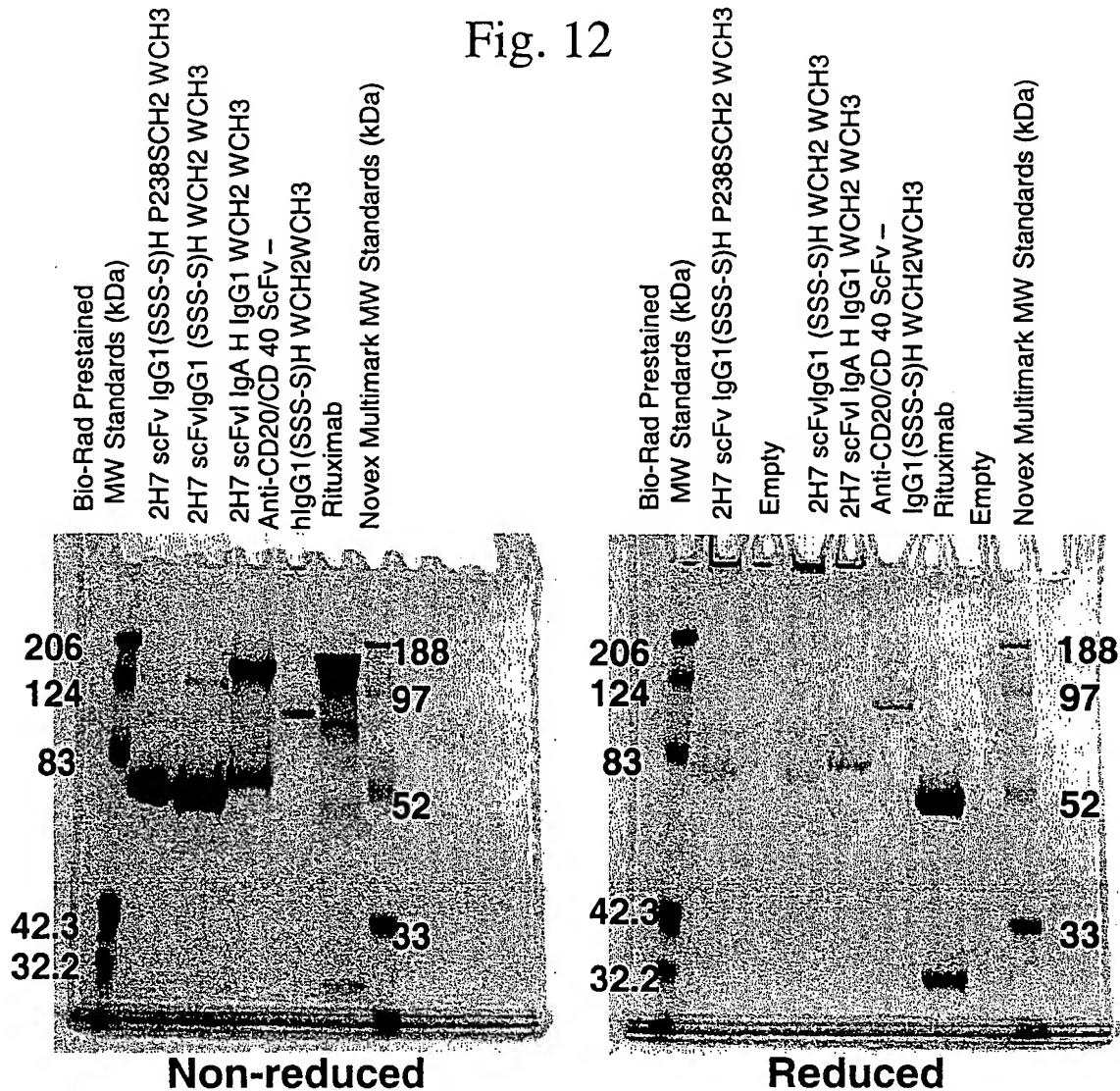


Figure 12: SDS-PAGE Analysis of CytoxB Derivatives. Purified fusion protein derivatives of CytoxB-scFvIg molecules and Rituximab were resuspended in SDS sample buffer, boiled, loaded onto 10% Novex Tris-Bis gels (Invitrogen, San Diego, CA) and subjected to nonreducing (left panel) or reducing (right panel) SDS-PAGE electrophoresis at 175 volts. Two different molecular weight markers, BioRad prestained markers, and Novex Multimark molecular weight markers were also loaded onto each gel and the approximate size in kDa of each marker band is indicated along each side of the photographed gels. Gels were stained in Coomassie Blue stain and photographed with a SONY Mavica Digital camera. The mutant hinge forms of 2H7 scFvIgG1 migrate at approximately 70 kDa under both nonreducing and reducing conditions, indicating that these molecules are monomeric rather than dimeric in structure. The IgA hinge form of 2H7scFvIg migrates at approximately 75 kDa under reducing conditions, but migrates predominately as a dimer of 140 kDa with a fraction of the protein migrating at 75 kDa under nonreducing conditions. Under nonreducing conditions, rituximab migrates as a diffuse band of between 150 and 200 kDa. The heavy and light chains resolve into separate bands of approximately 32 and 50 kDa when rituximab is reduced and subjected to SDS-PAGE.

Fig. 13
ADCC Activity of Cytox B (2H7 scFvIg) Constructs.

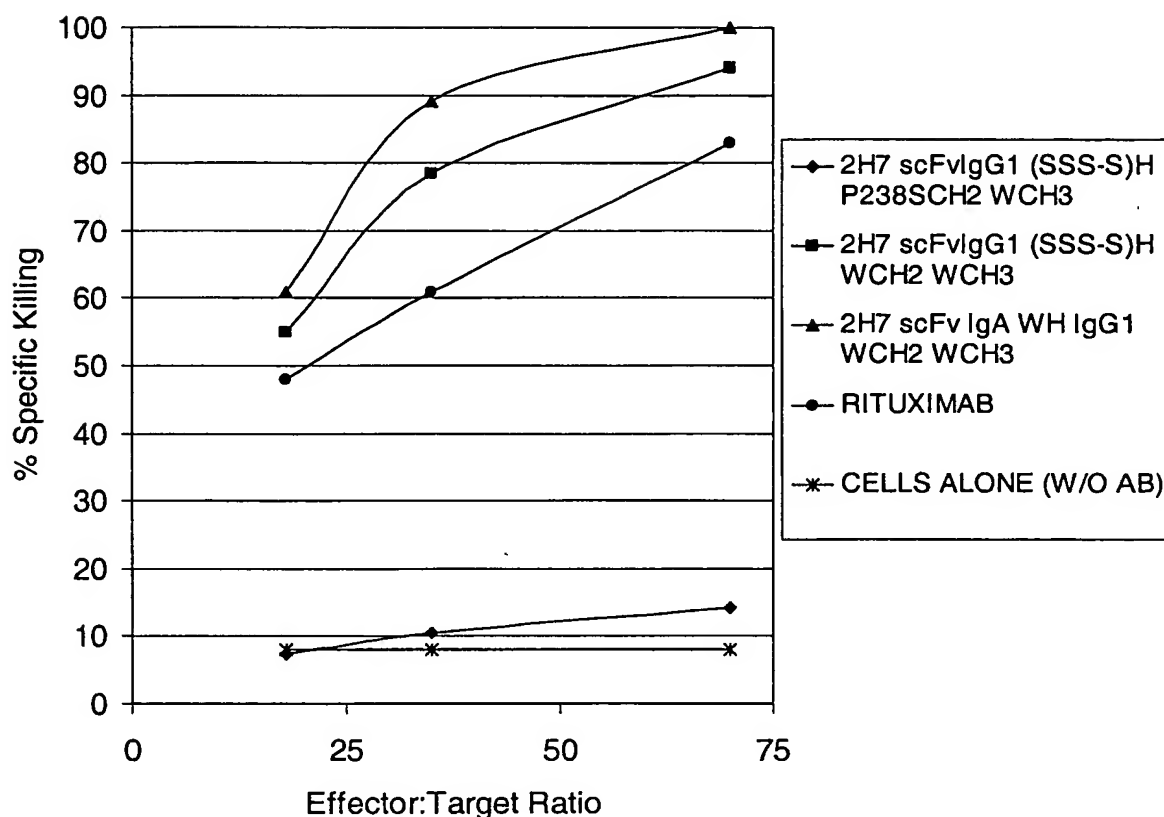


Figure 13: ADCC Activity of Cytox B Derivatives Compared to Rituximab. ADCC activity of Cytox B Derivatives or Rituximab was measured *in vitro* against BJAB B lymphoma cell line as target and using fresh human PBMC as effector cells. Effector to target ratios were varied as follows: 70:1, 35:1, and 18:1, with the number of BJAB cells per well remaining constant but varying the number of PBMC. Bjab cells were labeled for 2 hours with ^{51}Cr and aliquoted at a cell density of 5×10^4 cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or rituximab were added at a concentration of 10 mg/ml, and PBMC were added at 9×10^5 cells/well (18:1), 1.8×10^6 cells/well (35:1), or 3.6×10^6 cells/well (70:1), in a final volume of 200 μl . Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 4 hours, and 100 μl culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter.

Fig. 14
CDC of Cytos B (2H7 scFvIg) Constructs

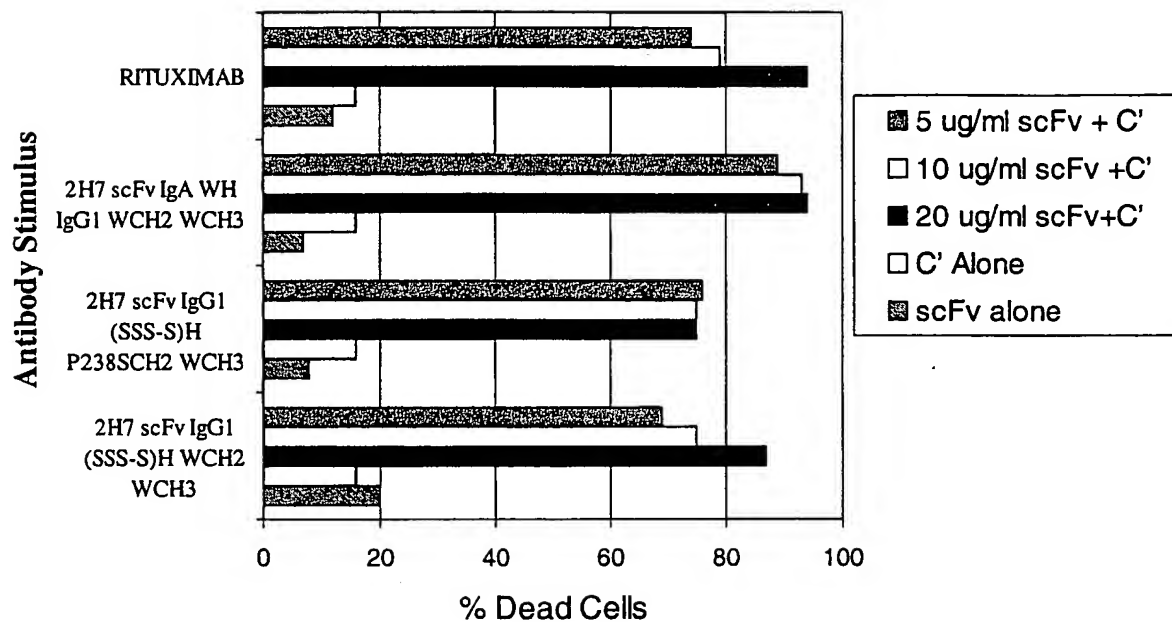
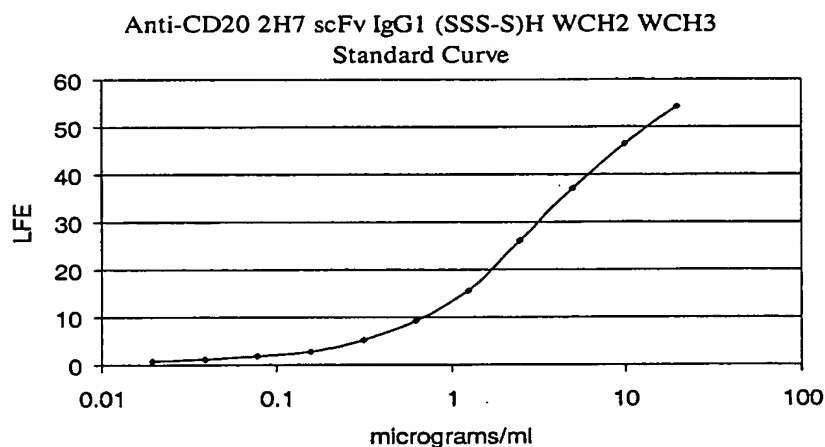


Figure 14: Complement Dependent Cytotoxicity (CDC) Activity of CytosB Derivatives Compared to Rituximab. 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, and 2H7scFv IgA WH IgG1 WCH2 WCH3 derivatives and Rituximab were compared for their ability to mediate complement dependent cytotoxicity. Rabbit complement (Pel-Freez) was diluted 1:10 and added to BJAB cells along with dilutions of each antibody derivative (20 μ g/ml, 10 μ g/ml, and 5 μ g/ml). Controls were also included without addition of complement (C') or scFv derivative. Reactions were allowed to continue for 1 hour, and cells from each well were then stained with trypan blue and the cell viability counted using a hemacytometer. Data is graphed as % of dead cells/total cells counted for each condition assayed.

Fig. 15
2H7 (anti-CD20) scFv IgG1 (SSS-S)H WCH2 WCH3
In Vivo Half Life



Macaque A99314

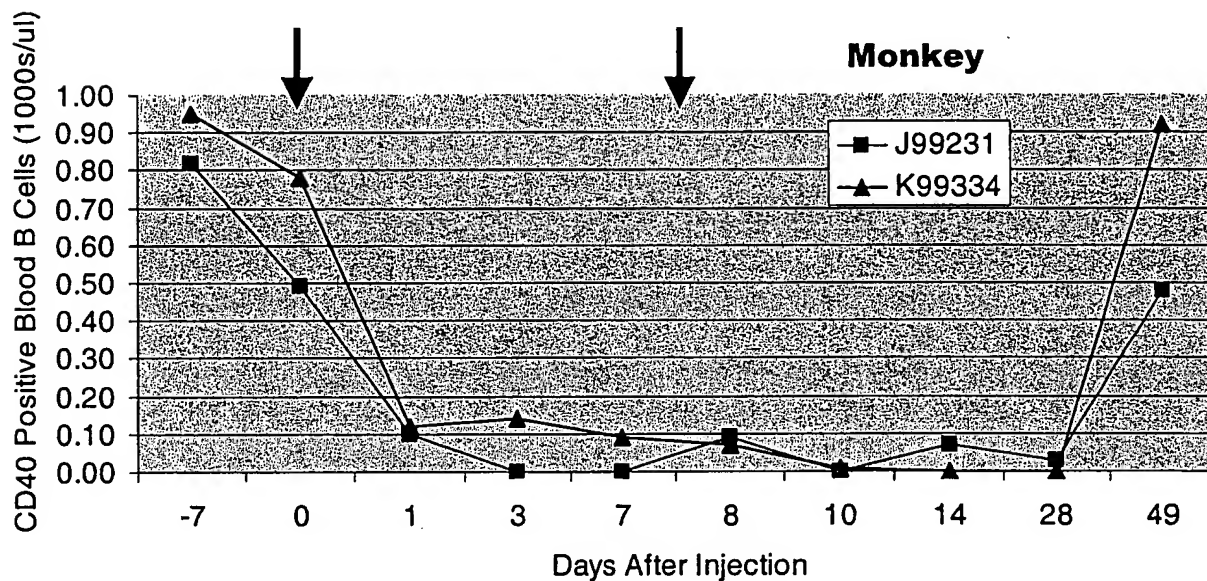
	Day	Binding intensity (LFE) @ 1:50 dilution of serum	estimated concentration (µg/ml)
Injection #1	-7	0.213	<0.1
	0	0.227	<0.1
	1	7.79	25.1
Injection #2	3	5.51	15.6
	7	3.37	9.4
	8	11.33	41.7
	10	5.45	15.4
	14	0.27	<0.1

Macaque F98081

	Day	Binding intensity (LFE) @ 1:50 dilution of serum	estimated concentration (µg/ml)
Injection #1	-7	0.208	<0.1
	0	0.219	<0.1
	1	6.73	21.9
Injection #2	3	6.14	19.3
	7	3.04	8.7
	8	9.83	33.8
	10	4.77	14.4
	14	0.231	<0.1

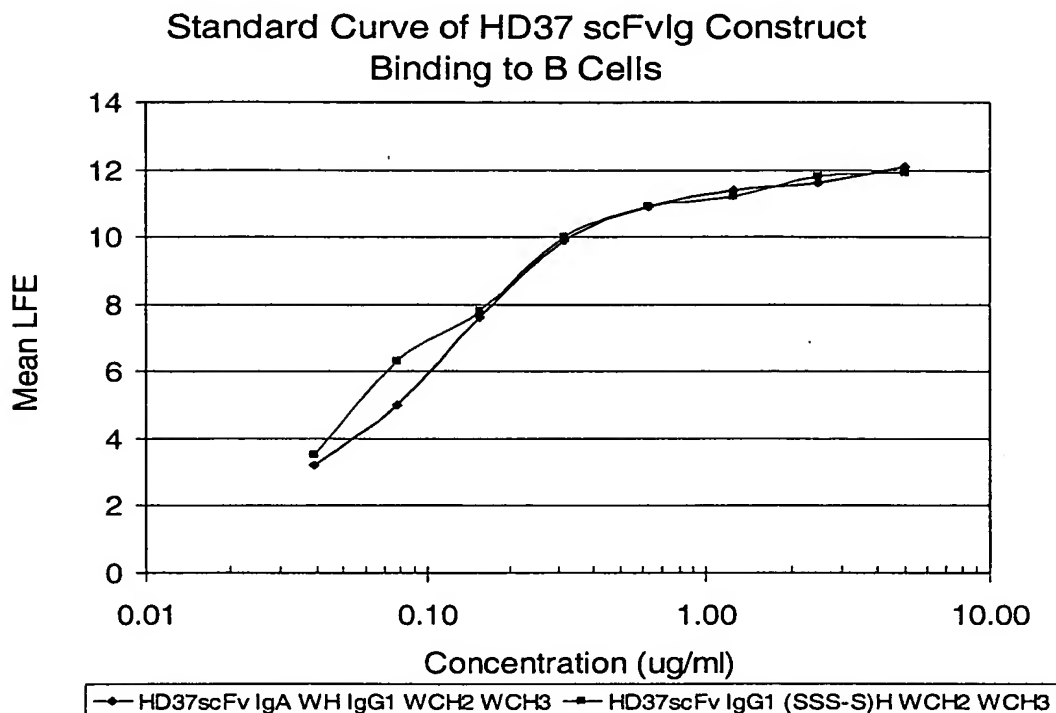
Fig. 16

B Cell Depletion in macaques mediated by Cytos B20
(2H7 scFv IgG1 (SSS-S)H WCH2 WCH3) Construct



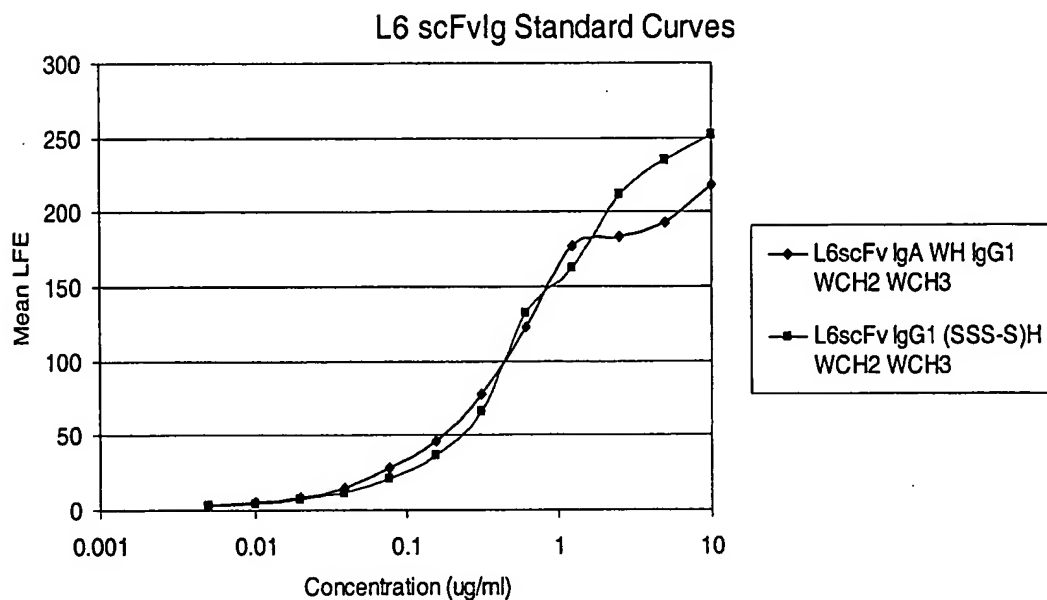
- CytosB20 injections of 6mg/kg yields 3 week B-cell depletion
- 3-4 day half-life *in vivo*
- CD20 saturation in lymph node B-cells at d14
- No first dose effects
- No anti-chimeric antibody development

Fig. 17
Production Levels of HD37 scFvIg Constructs
by CHO Cell Lines



Clone/Isolate	Mean LFE at 1:100	Estimated Concentration
Bulk HD37 scFv		
IgA WH IgG1 WCH2 WCH3	11.2	> 60 ug/ml
1B2	10.4	>50 ug/ml
6C5	10.5	>50 ug/ml
4B1	8.6	>40 ug/ml
Bulk HD37 scFv		
IgG1 (SSS-S)H WCH2 WCH3	10.9	> 50 ug/ml
2G8	10.6	> 50 ug/ml
3F3	8.3	>40 ug/ml
3D9	11.1	> 60 ug/ml

Fig. 18
Production of L6 scFvIg constructs by CHO Cells



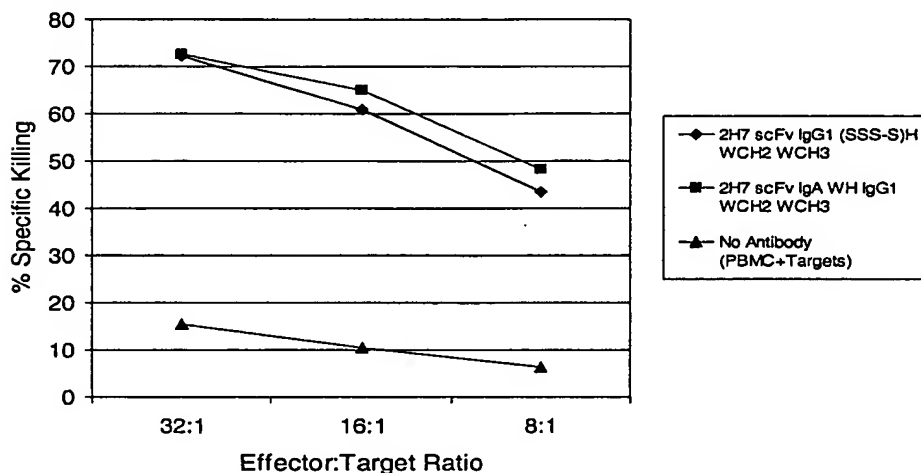
Construct	Mean LFE 1:20	Estimated Concentration
L6scFv IgA WH IgG1 WCH2 WCH3 unamplified CHO sup	51.1	6.25 ug/ml
L6scFv IgG1(SSS-S)H WCH2 WCH3 unamplified CHO sup	23.0	3.2ug/ml

Fig. 19

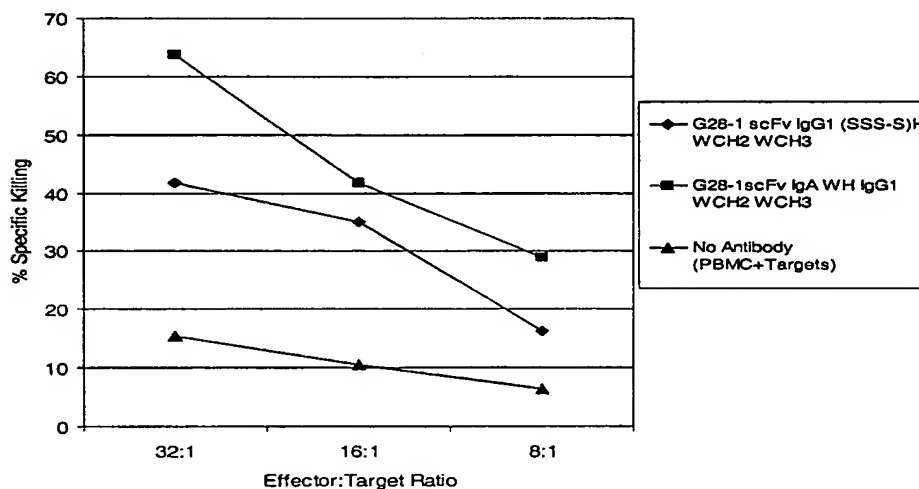
ADCC Activity of 2H7 scFvIg, G28-1 scFvIg, and HD37 scFvIg Constructs

ADCC Activity of scFvs Targeted to B Cell Antigens

A. 2H7 (anti-CD20) scFv constructs



B. G28-1 (anti-CD37) scFv constructs



C. HD37 (anti-CD19) scFv constructs

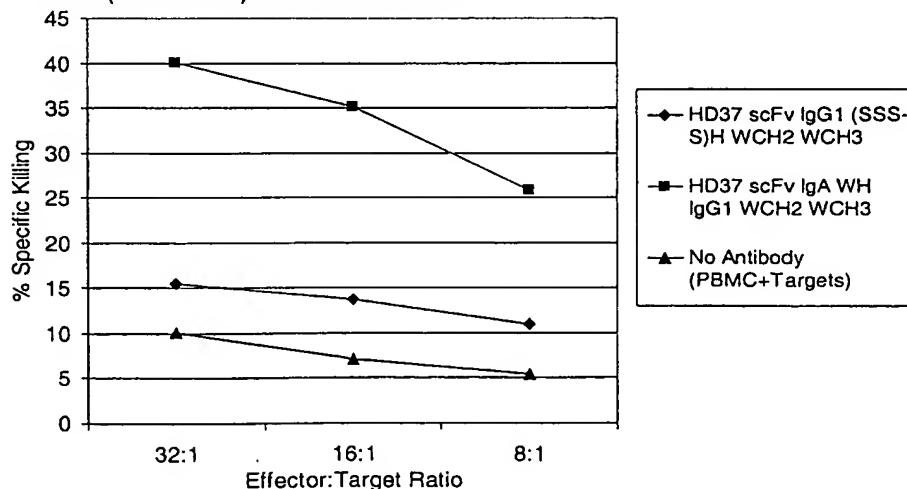


Fig. 20
ADCC Activity of L6 scFvIg Constructs

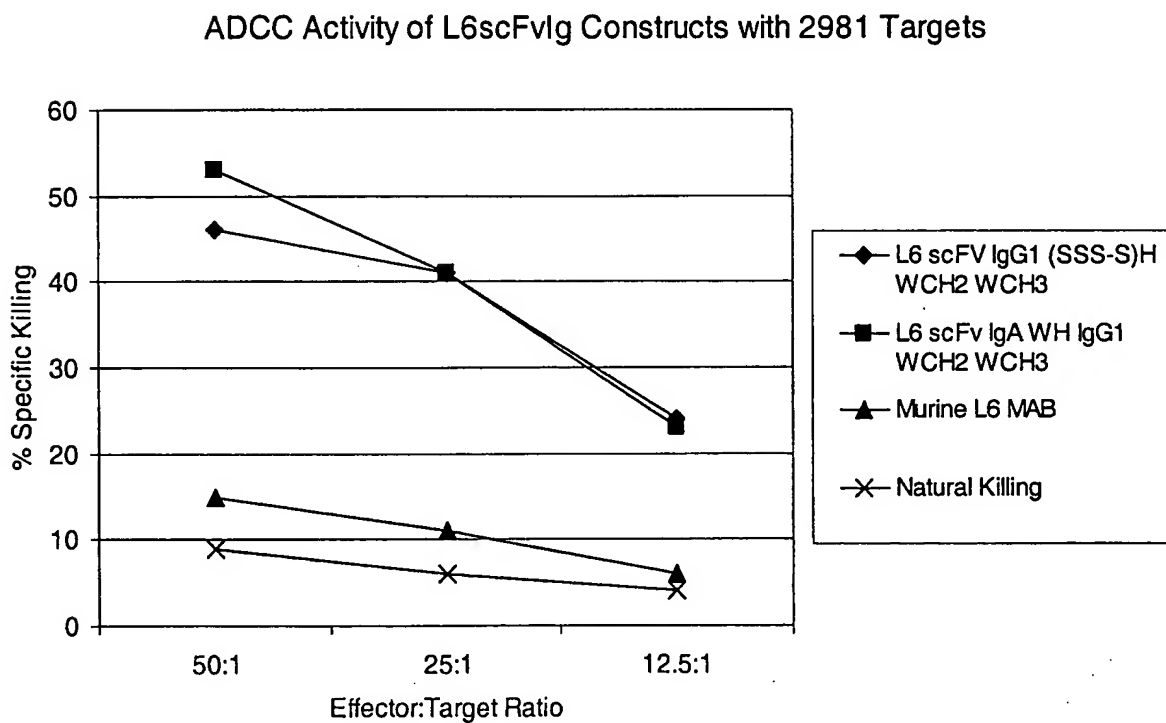


Fig. 21
SDS-PAGE Analysis of L6 and 2H7
scFvIg Fusion Proteins.

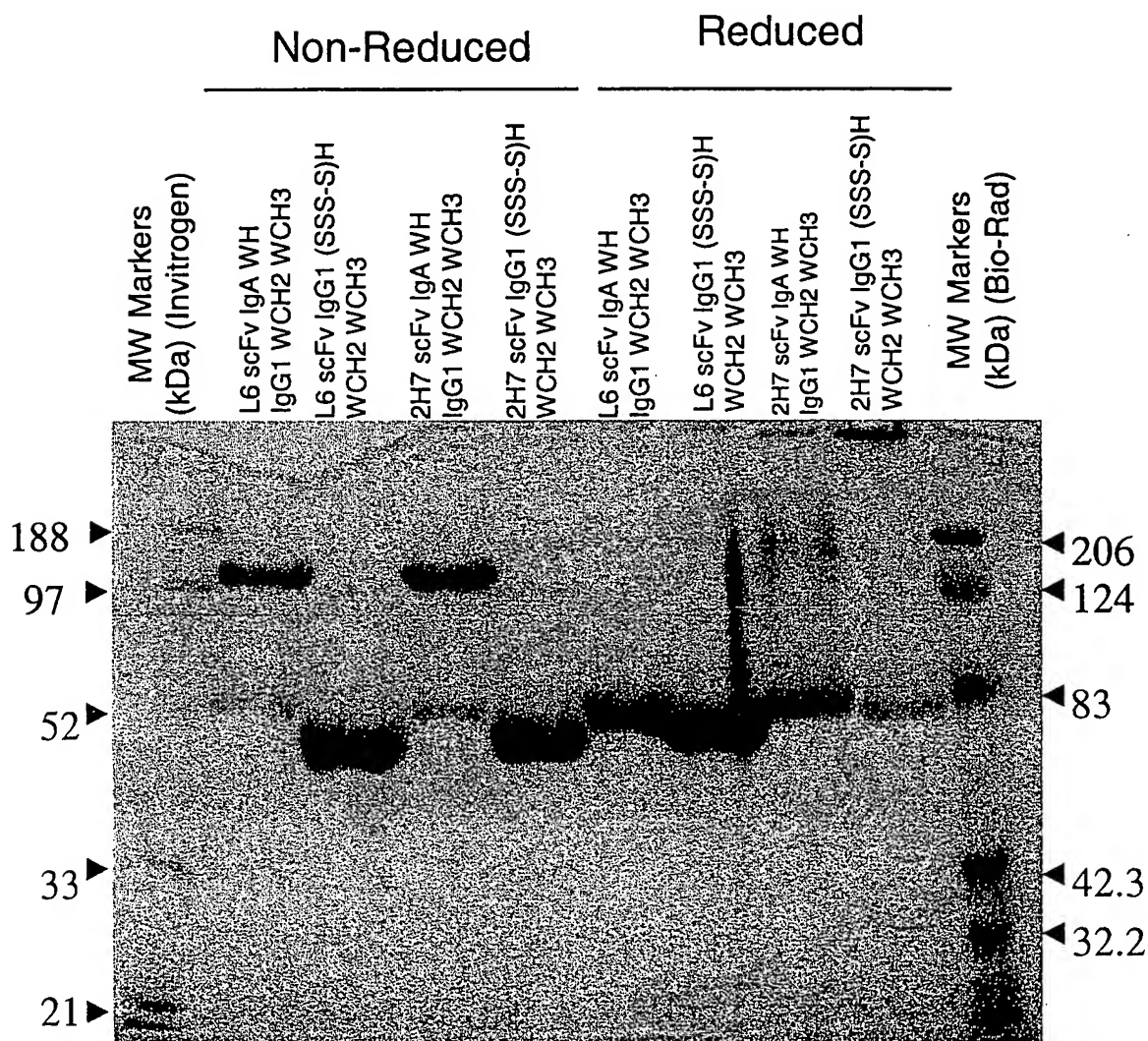


Fig. 22
SDS-PAGE Analysis of G28-1 and HD37
scFvlg Constructs.

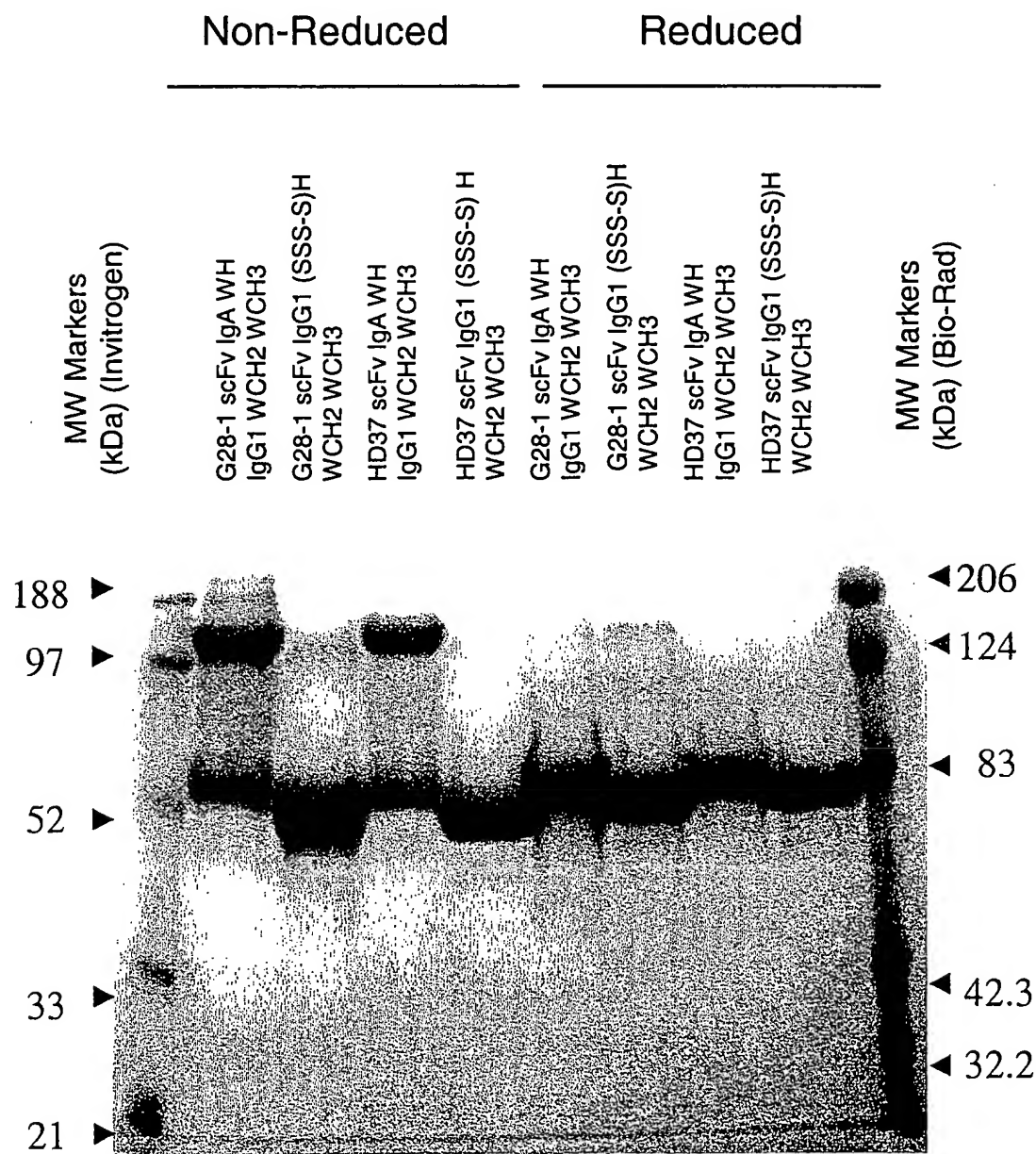


Fig. 23
Sequence alignment of human and llama Fc regions.

	HINGE	CH2→
Human IgG1:	DQEPKSCDKT-----HTCPPC	PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
Llama IgG2:	DQEPKTPKPQPQPQPQPNPTTESKCPKC	PAPELLGGPSVFIFFPKPKDVLISIGRPEVTCVVVDVGQEDPEVSFNWYIDG
Llama IgG1:	--EPHGG-----CTCPQC	PAPELPGGPSVFVFPPKPKDVLISIGRPEVTCVVVDVGKEDPEVNFNWIYIDG
Llama IgG3:	--AHHSEDPT-----SKCPKC	PGPELLGGPTVFIFPPKAKDVLISITRKPEVTCVWWTWVKKTLRSSSSWSVDD

VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
TAEVRANTRPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIEKTISKAKGQTPREPQVYTLAPHREELAKDTVSVT
VEVRTANTKPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTPREPQVYTLAPHREELAKDTVSVT
TEVHTAETKPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTPREPQVYTLAPHREELAKDTVSVT

CLVKGFYPSDIAVEWESNGQPEN--NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK
CLVKGFYPDPINVEWQRNGQPESXGTYATTPQLDNDGTYFLXSKXSVGKNTWQQGETFTCVVMHEALHNHYTQKSITQSSGK
CLVKGFYPADINVEWQRNGQPESEGTYANTPPQLDNDGTYFLYSLVSGKNTWQRGETLTGVVMHEALHNHYTQKSITQSSGK
CLVKGFFPADINVEWQRNGQPESEGTYANTPPQLDNDGTYFLYSLVSGKNTWQQGEVFTCVVMHEALHNHSTQKSITQSSGK

Fig. 24

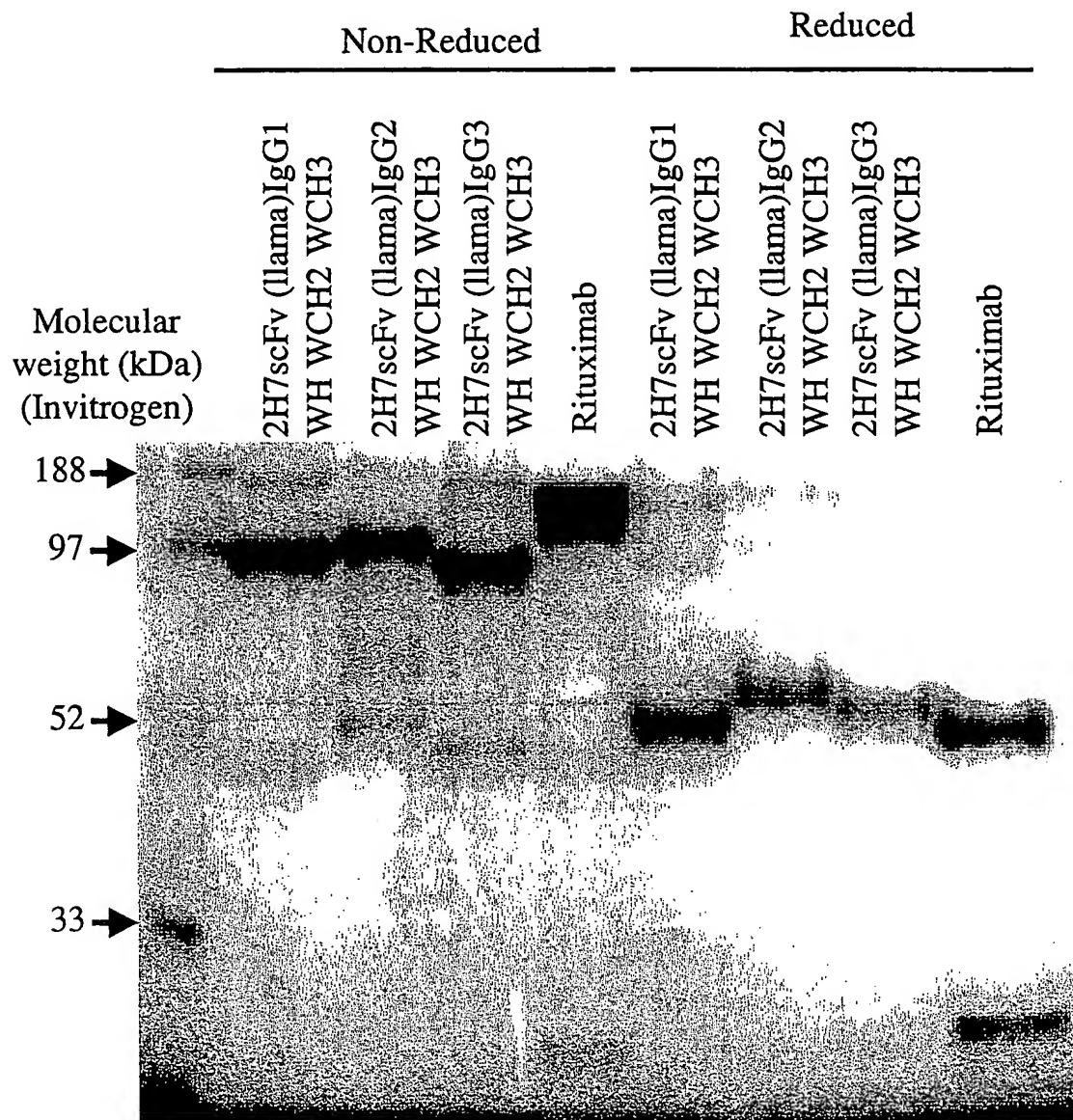


Fig. 25

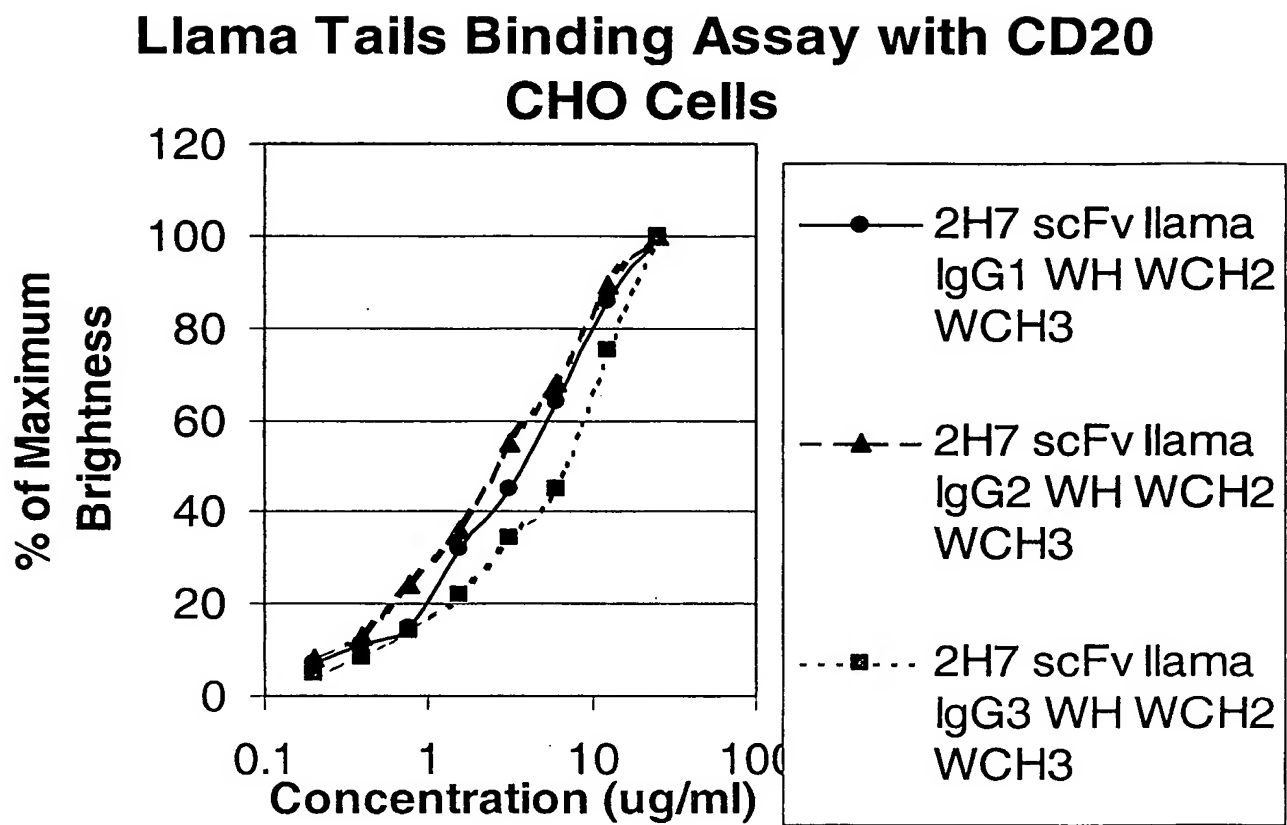
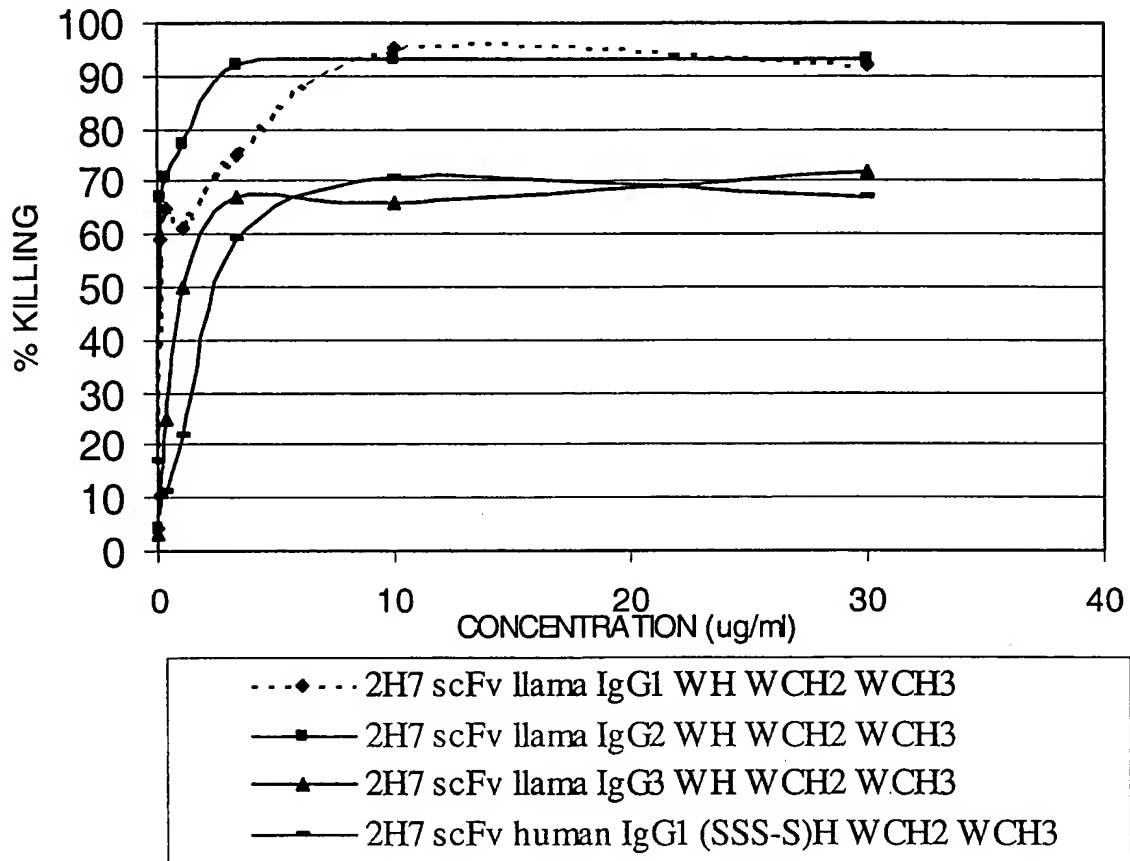


Fig. 26

2H7 scFvIg Llama Tails binding Assay with CD20 CHO Cells



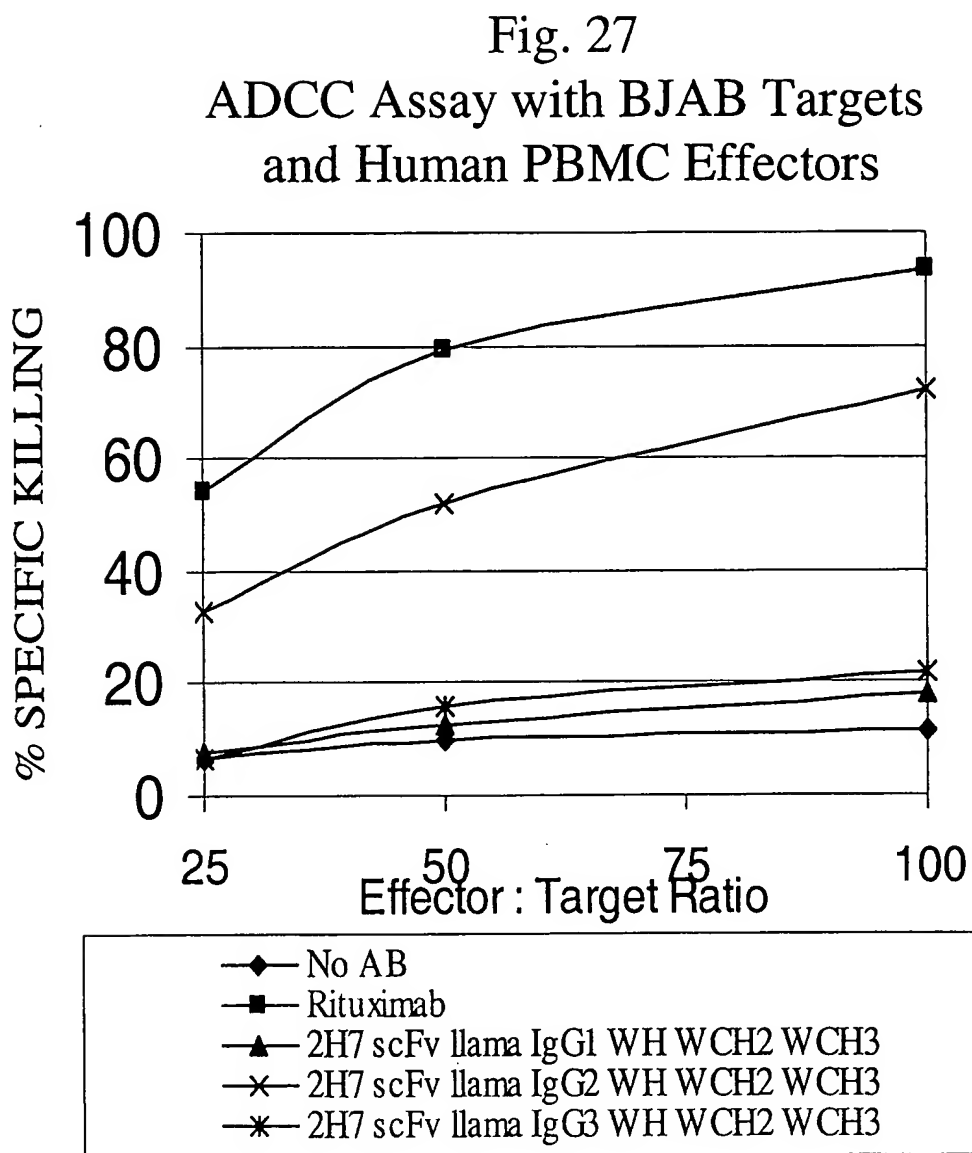


Fig. 28

ADCC Assay with BJAB Cells
And Llama PBMC Effectors

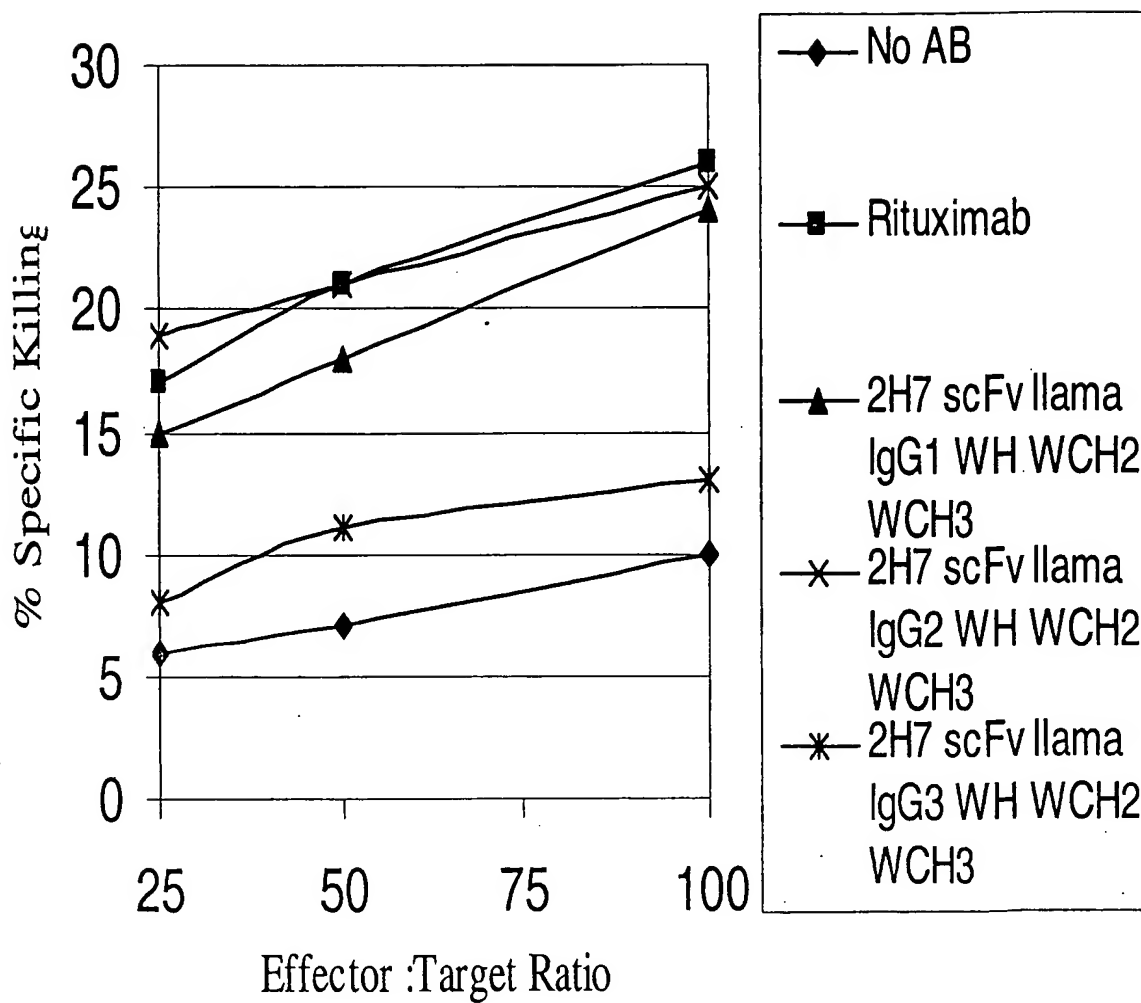


Fig. 29

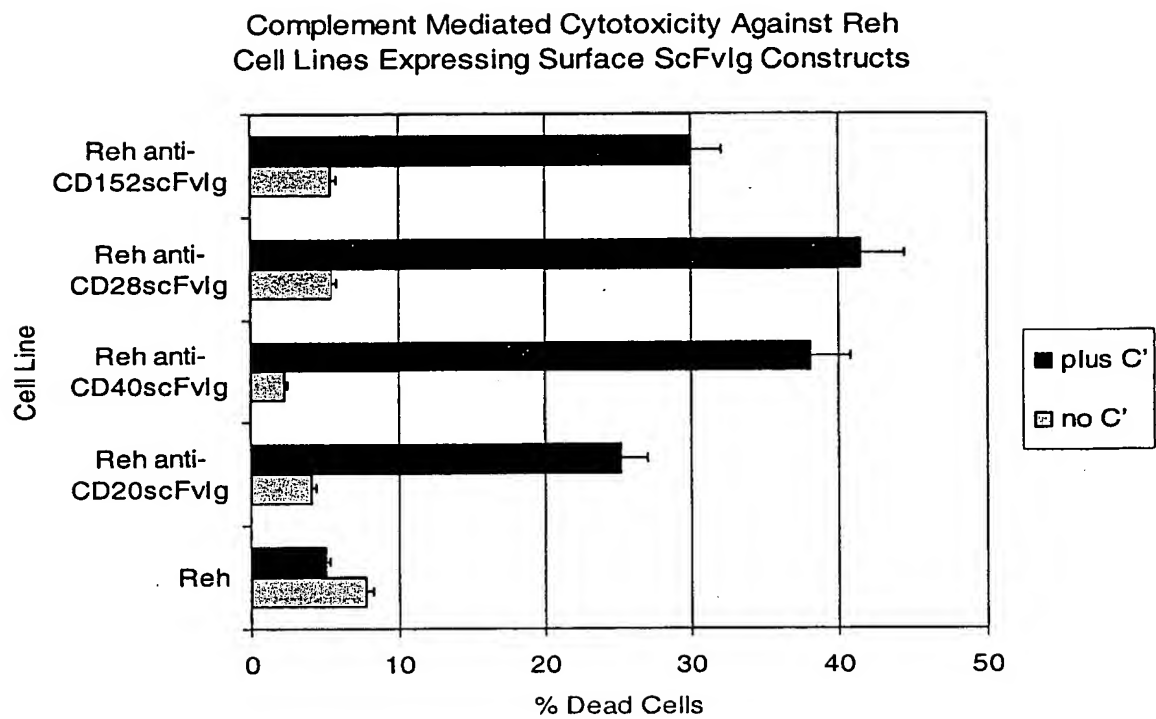


Fig. 30

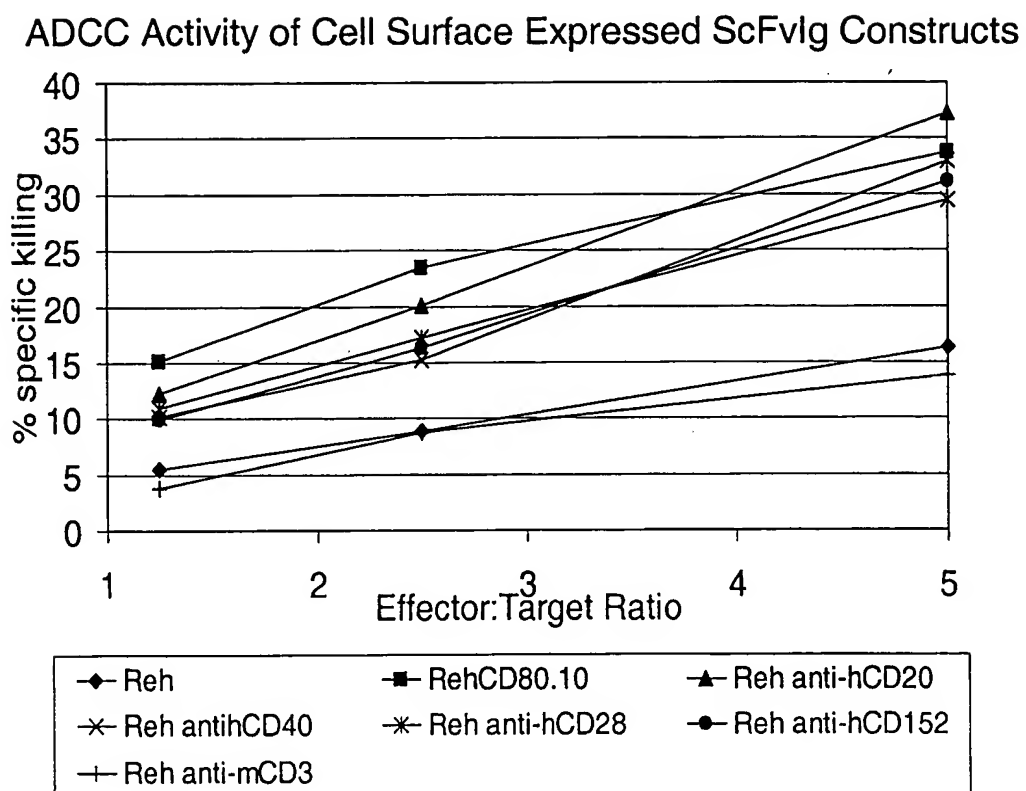


Fig. 31
Ig Constructs and Nomenclature:

Name Identifier	Hinge Sequence	CH2 Sequence	CH3 Sequence
hIgG1 (CCC-P)H WCH2 WCH3	IgG1 WT Hinge (CCC-P)	Wild Type CH2	Wild Type CH3
hIgG1 (SSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
VH L11S hIgG1 (SSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (SSC-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSC-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (SCS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SCS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (CSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (CSS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (SSS-S)H P238S CH2 WCH3	IgG1 Mutant Hinge (SSS-S)	Mutant CH2 (IgG1) Pro→Ser 238	Wild type CH3 (IgG1)
IgA WH hIgG1 WCH2 WCH3	IgA Hinge	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgA WH IgA WCH2 WCH3	IgA Hinge	Wild type CH2 (IgA)	Wild type CH3 (IgA)
IgA WH IgA WCH2 T4CH3	IgA Hinge	Wild type CH2 (IgA)	Truncated CH3 (IgA) Missing 4 aa at COOH

Fig. 32

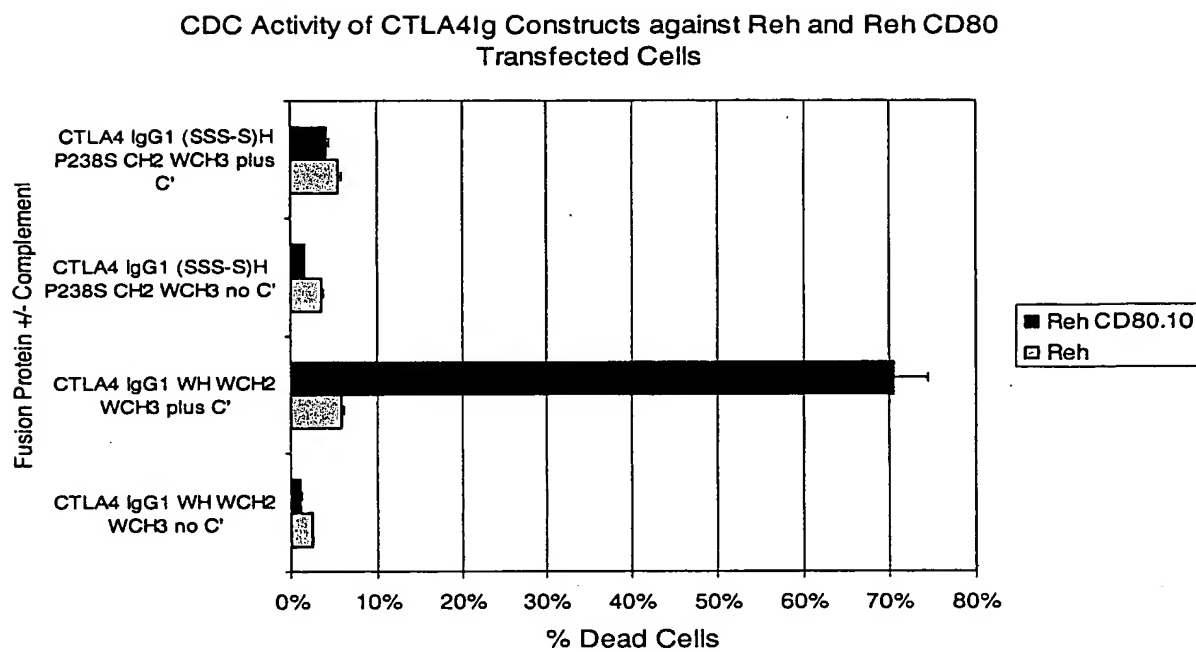


Fig. 33

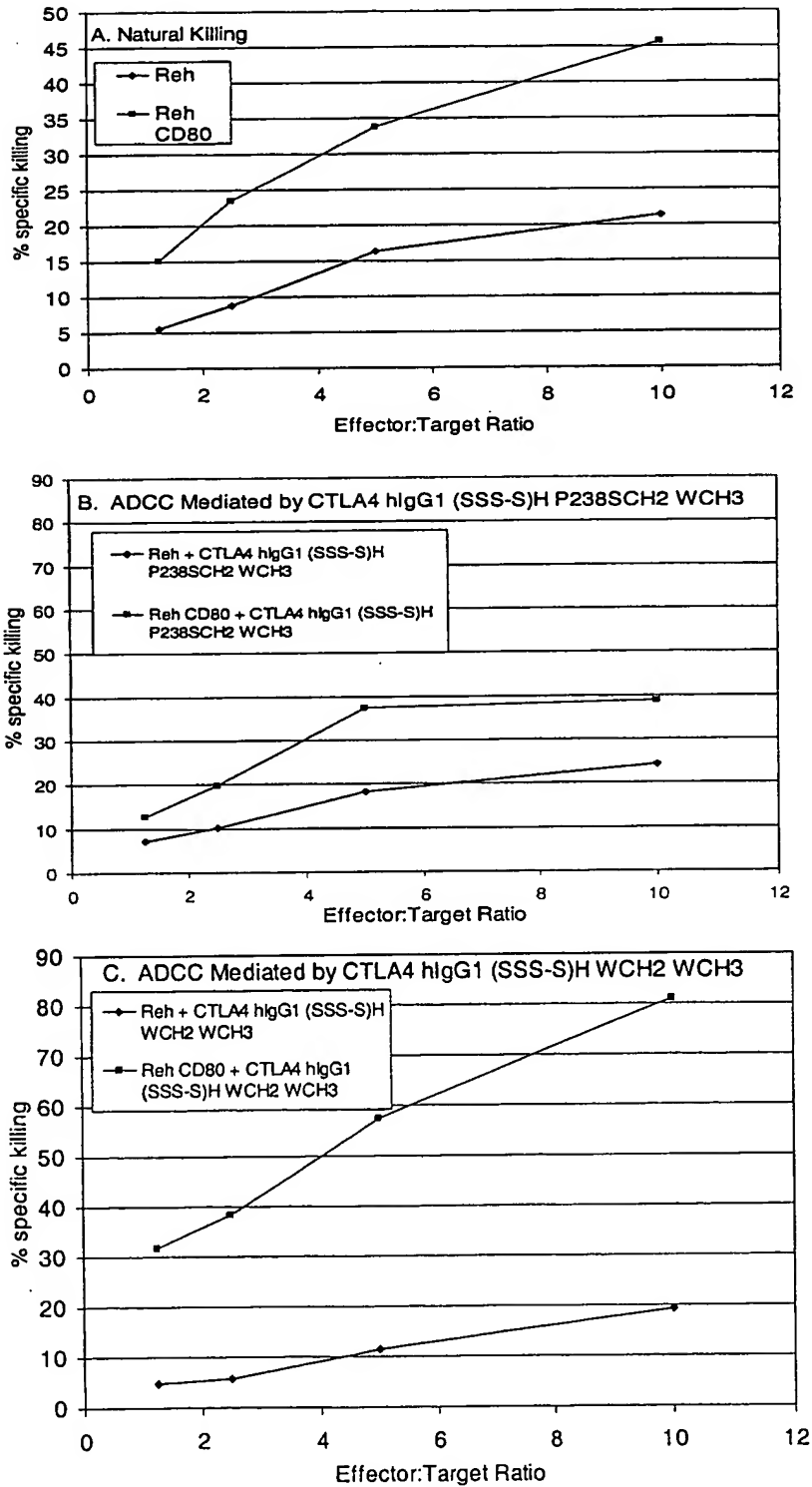


Fig. 34

Binding of 2H7 scFvIg Constructs
with Alternative Tails to CD20 CHO Cells

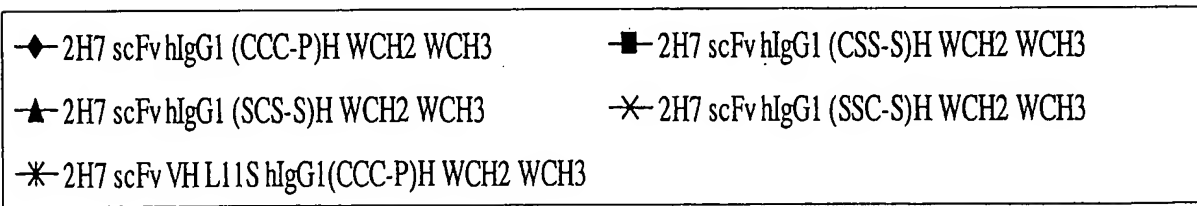
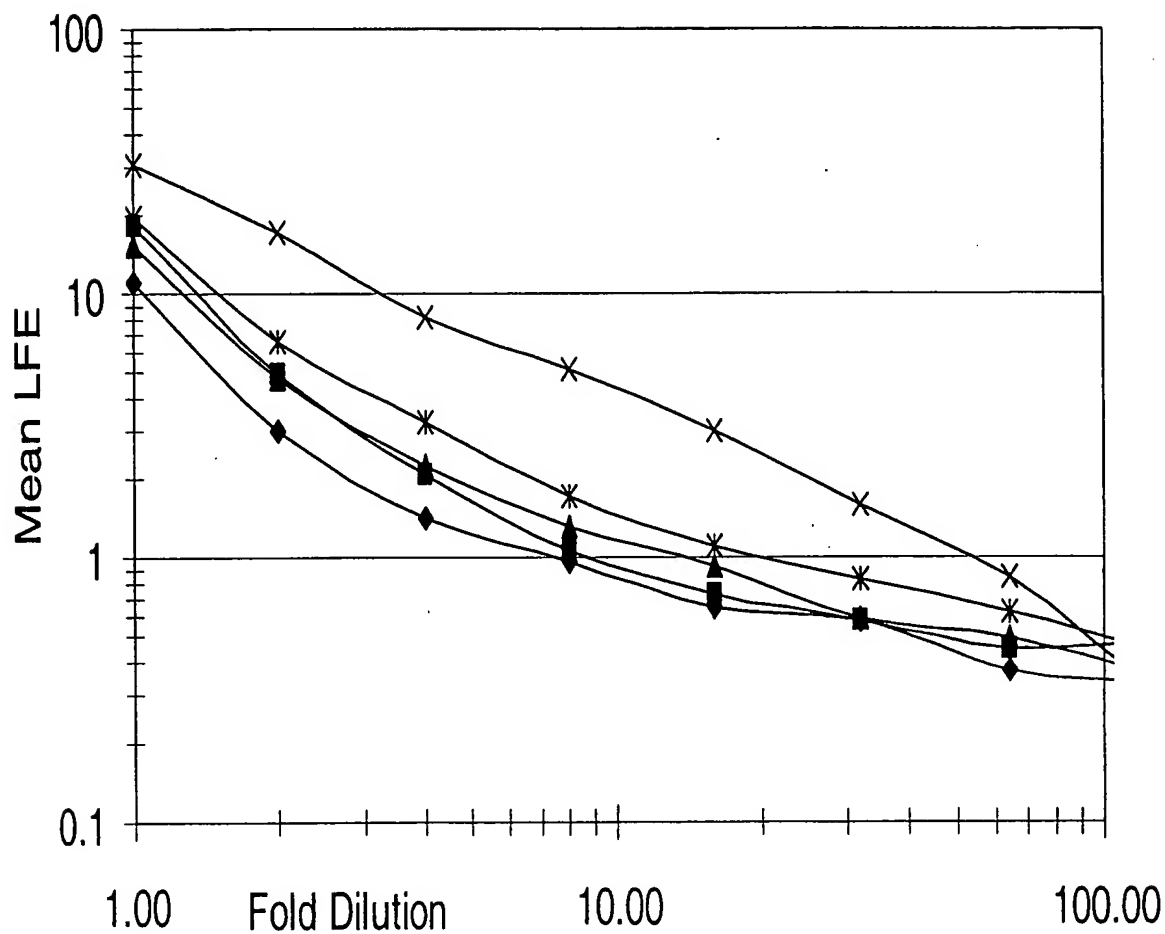


Fig. 35
Immunoblot Analysis of protein immunoprecipitates
from COS transfections of 2H7 scFvIg Constructs

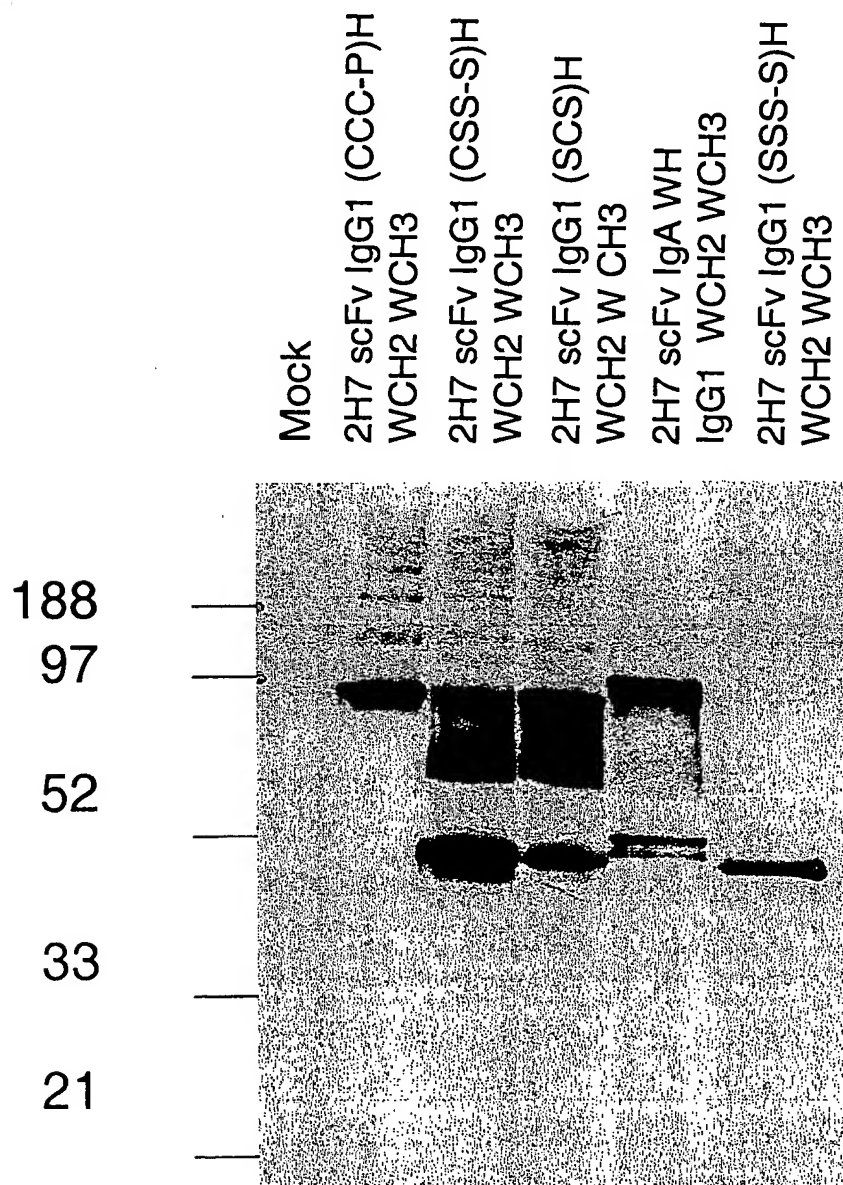


Fig. 36

Binding to CD20 CHO cells by constructs
that link anti-CD20 scFv to IgA Fc Domains

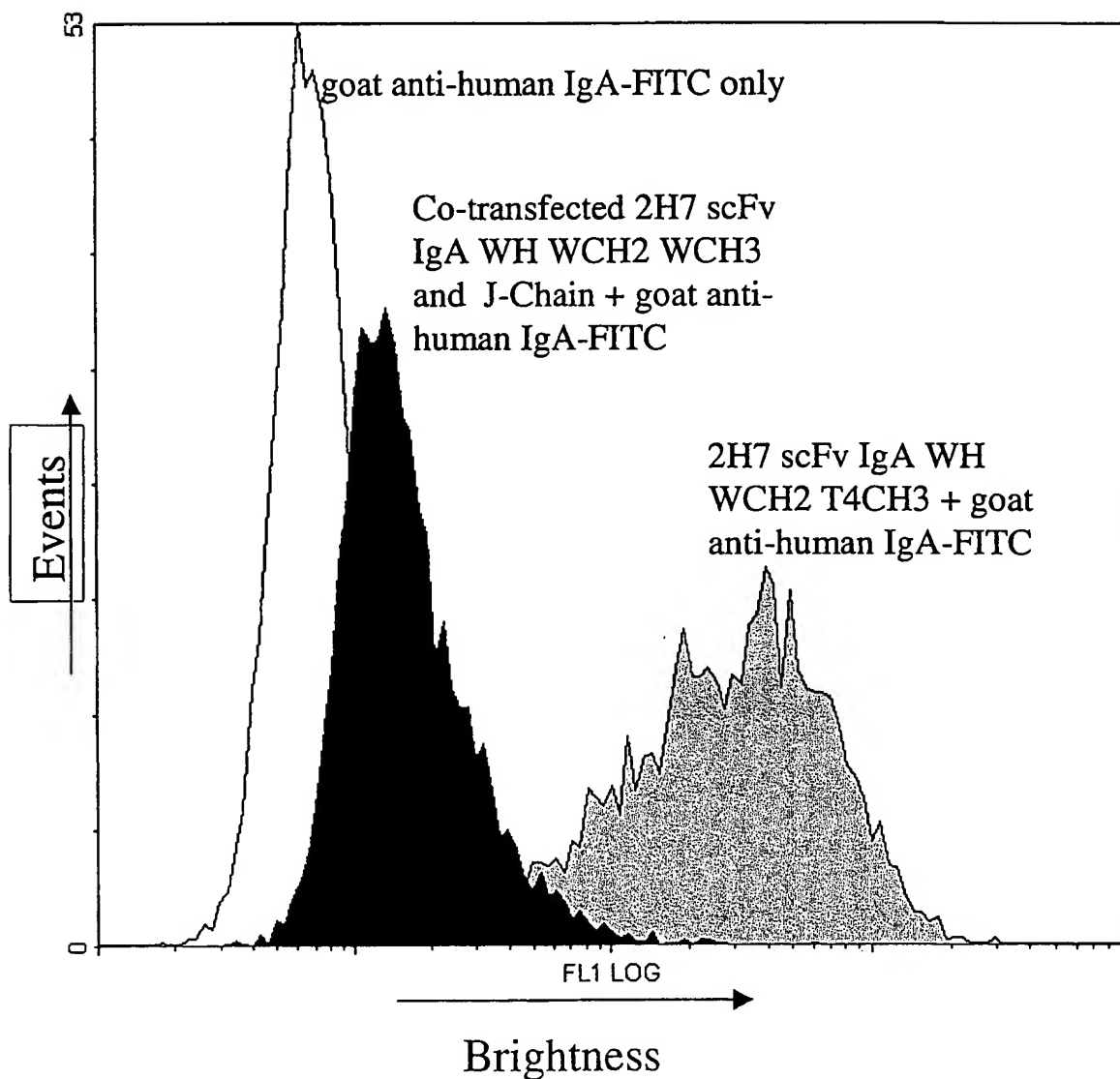


Fig. 37

Titration of CD20 specific scFvIg Constructs
for ADCC Activity Using Whole Blood Effectors

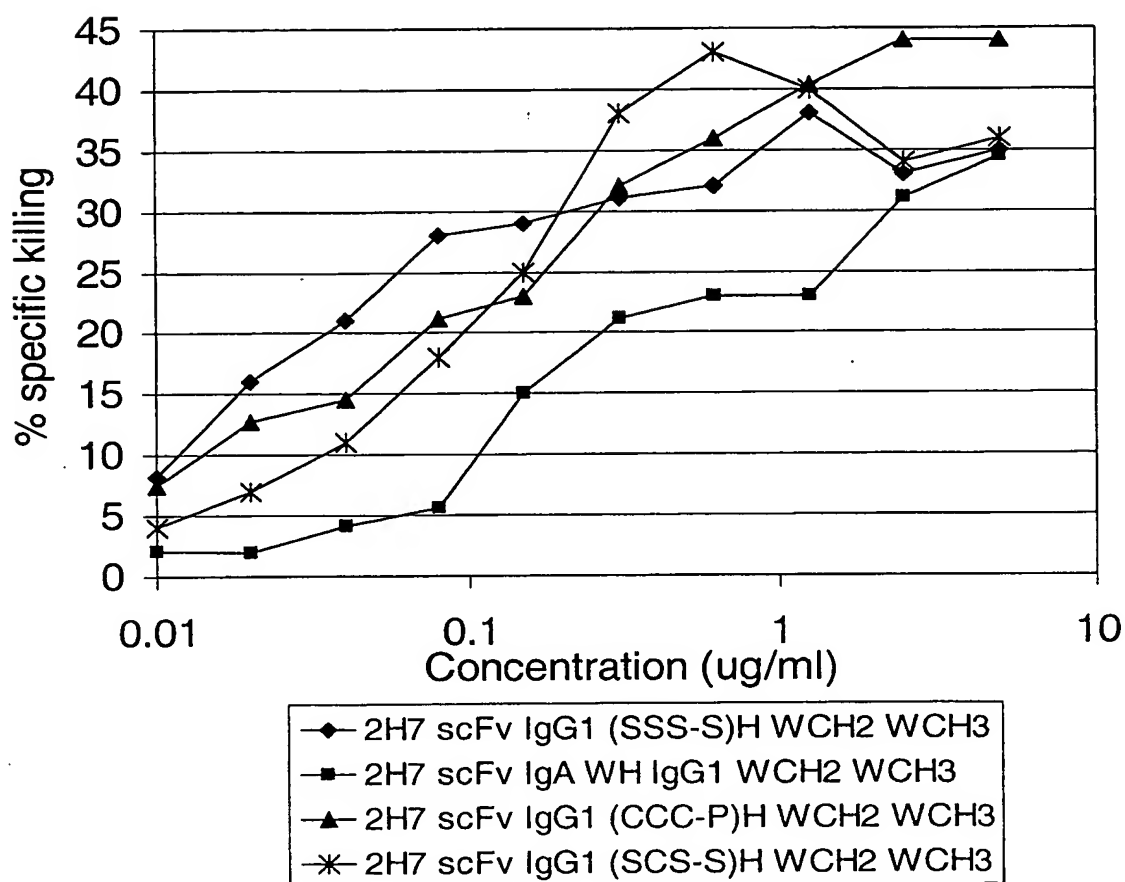


Fig. 38

ADCC Assay of anti-CD20 constructs with alternative tails
(Whole Blood Effectors / BJAB Targets)

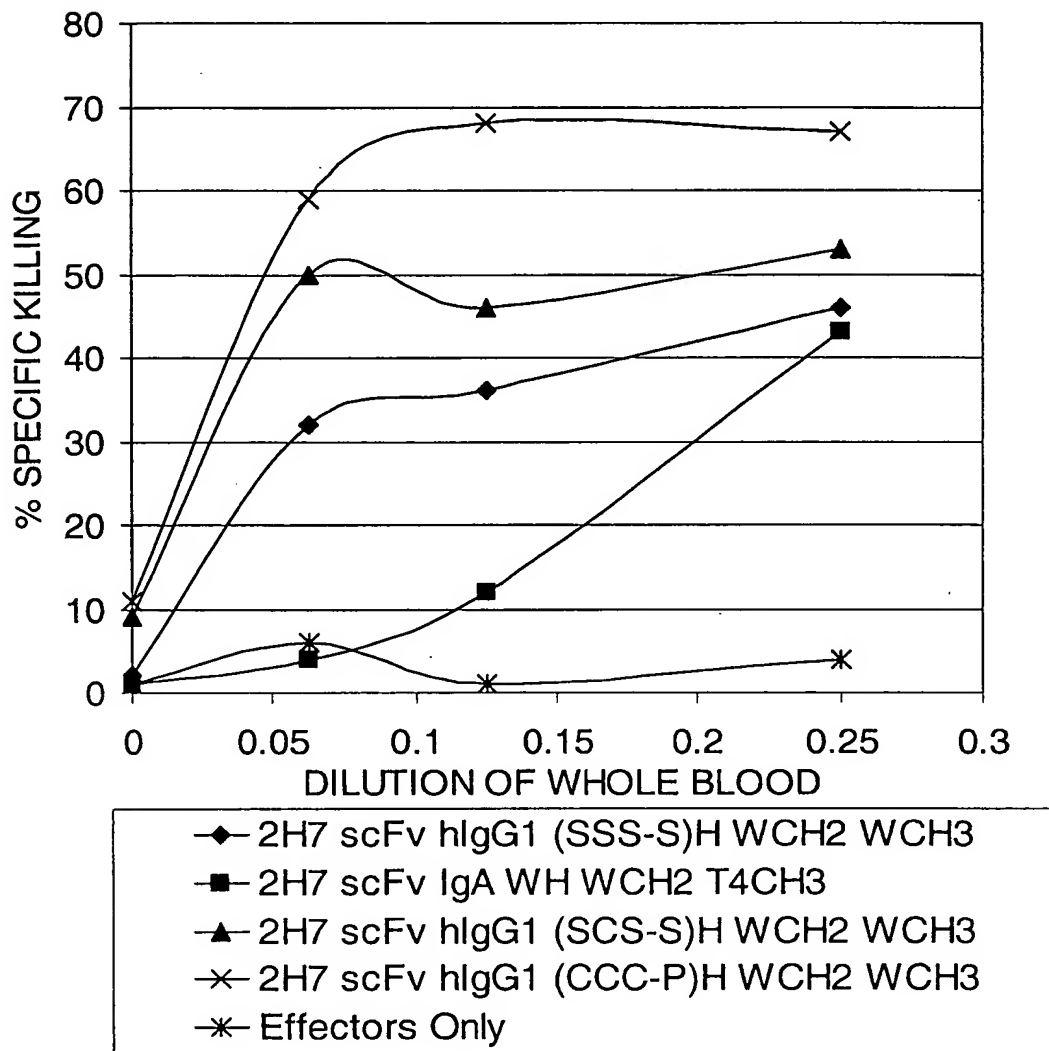


Fig. 39

ADCC Assay of Anti-CD20 scFvIg Constructs
Using Different Effector Populations Against BJAB Targets

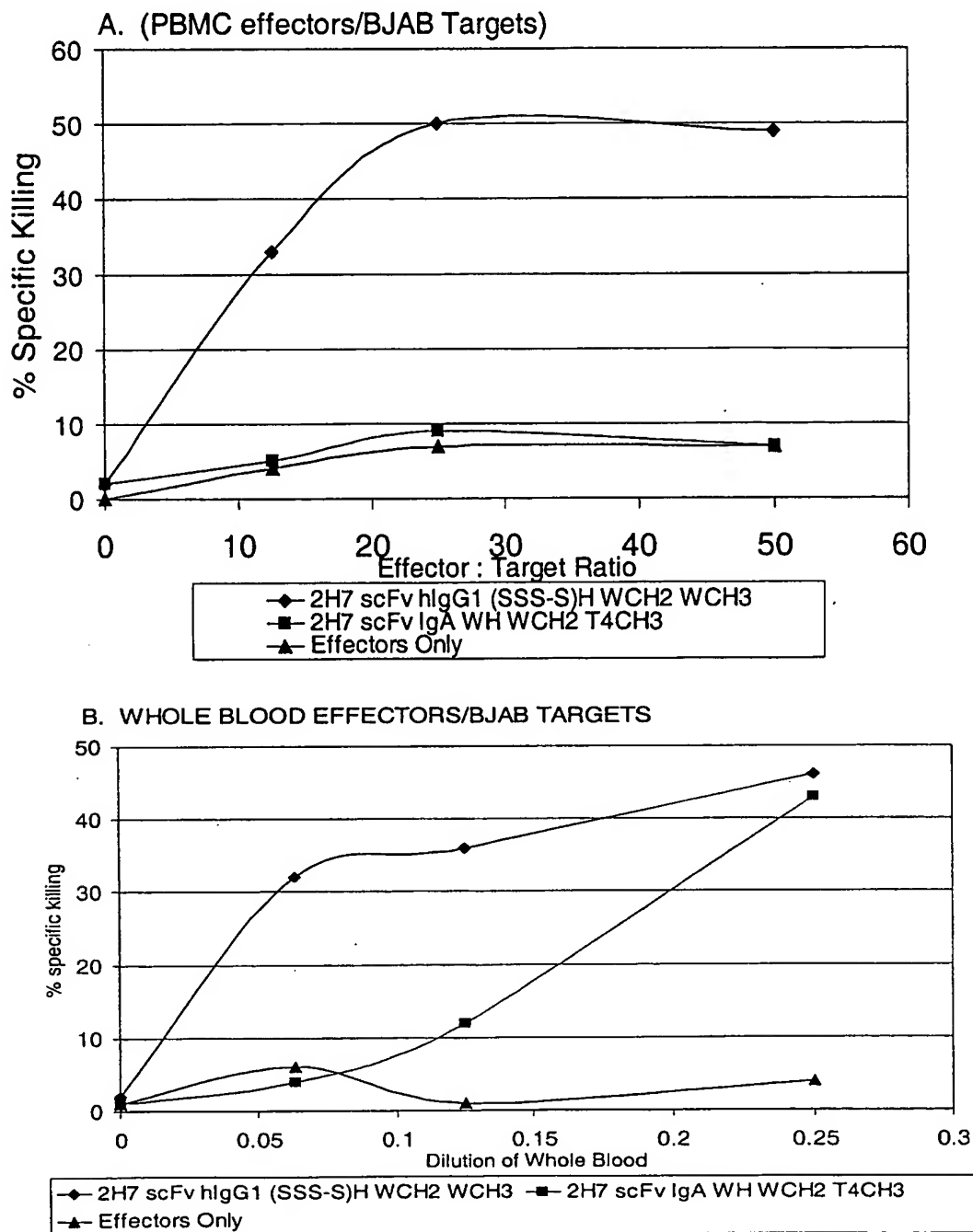
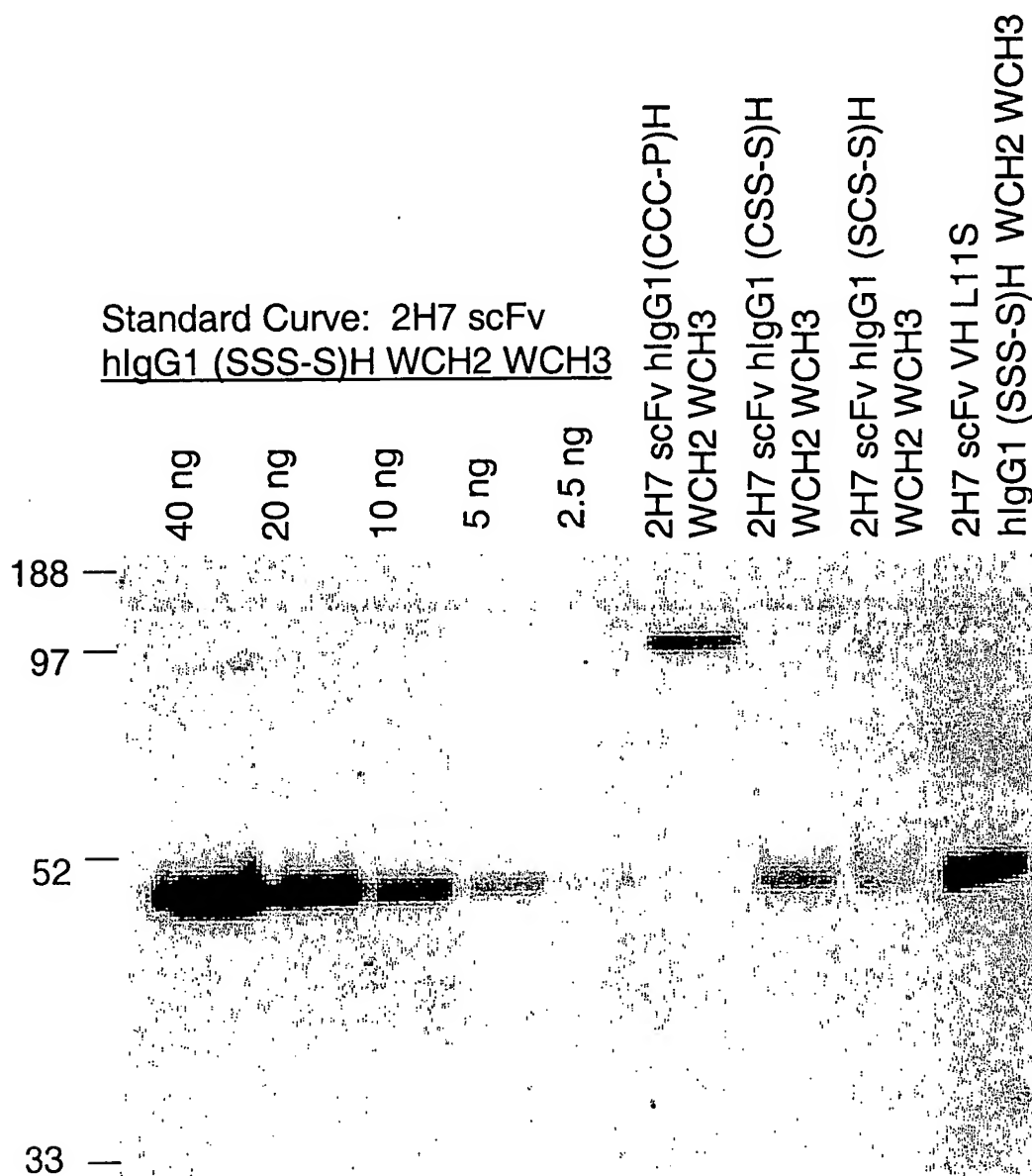


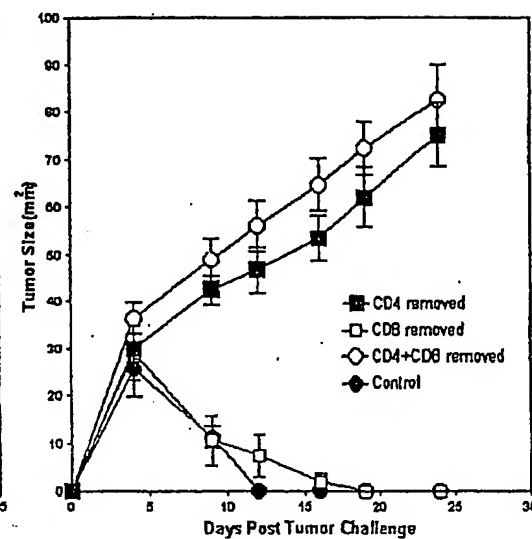
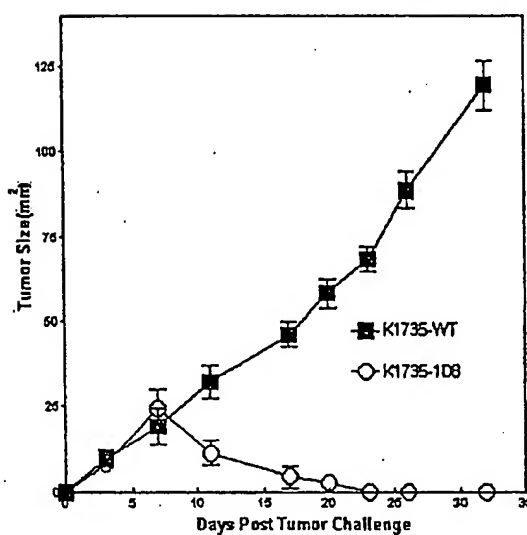
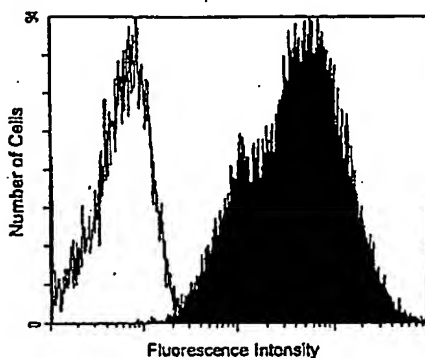
Fig. 40

Immunoblot of 2H7 scFv Ig constructs from COS
Transfections (1 μ l/well) compared to a Concentration Standard



Figures 41A, 41B and 41C

A.



Sheet 45 of 53

B.

C.

Fig. 42

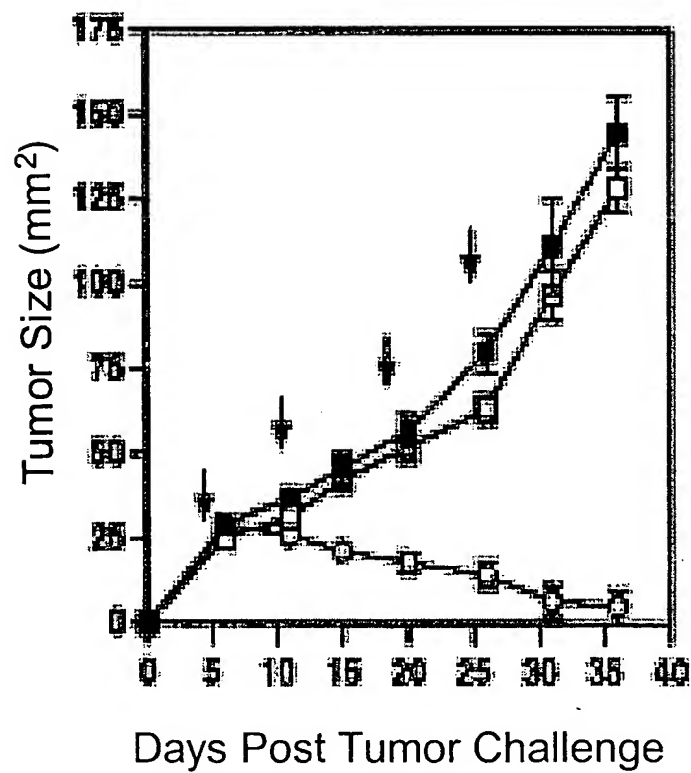


Fig. 43

Mixtures of K1735-WT and K1735-1D8 transfected tumor lines
inhibit tumor outgrowth in C3H mice

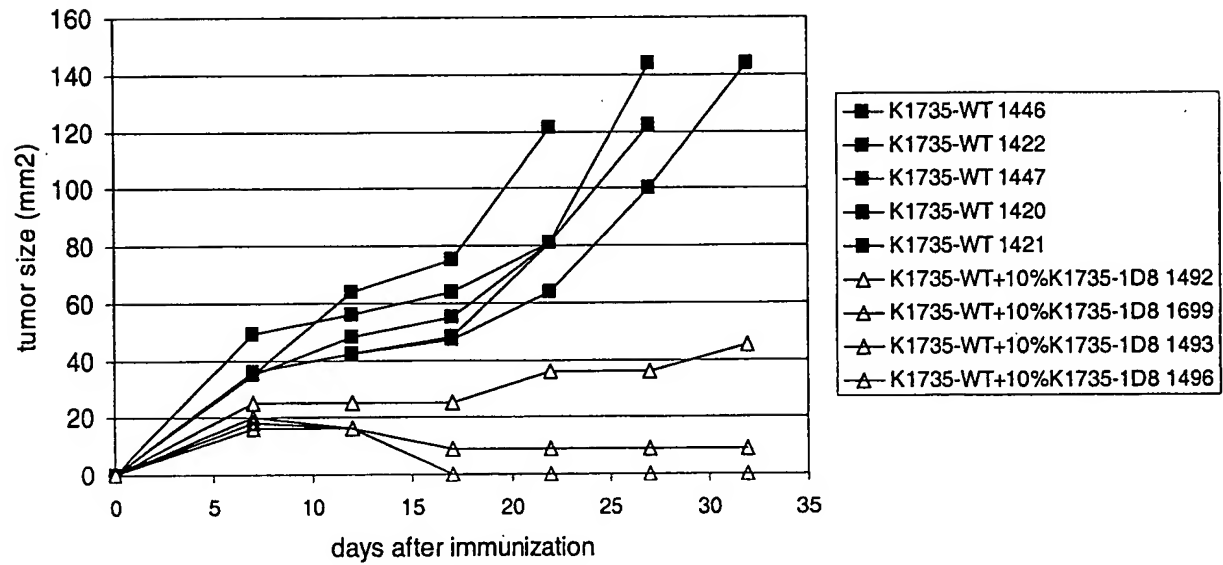


Fig. 44

Expression of anti-mouse CD137 (1D8) scFv-hIgG1 (SSS-S)H P238SCH2 WCH3
On the surface of panned Ag104-1D8 Transfected Tumor Cells

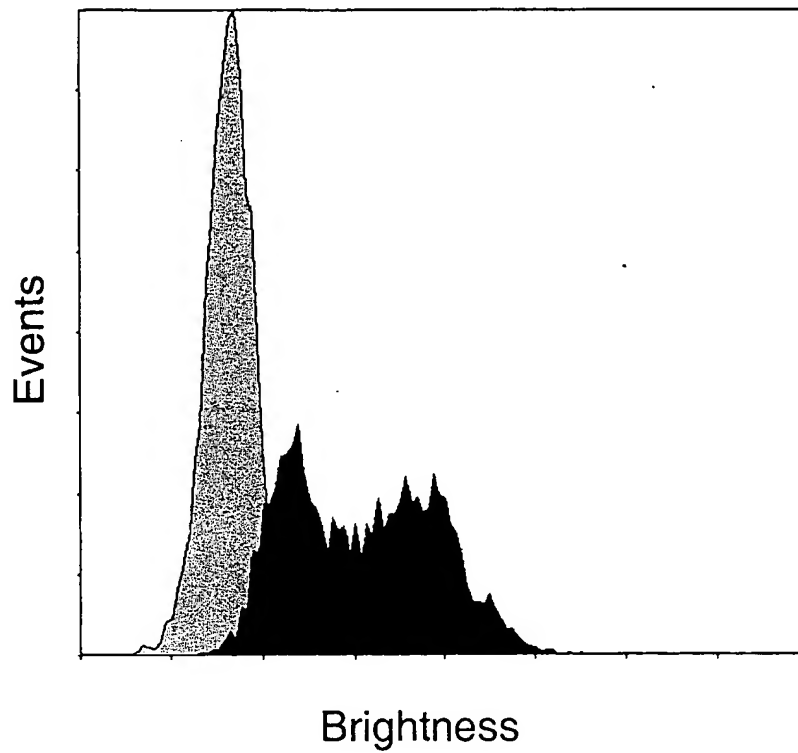


Fig. 45
Coomassie Stained SDS-PAGE Gel of 2H7 scFv Ig
Constructs

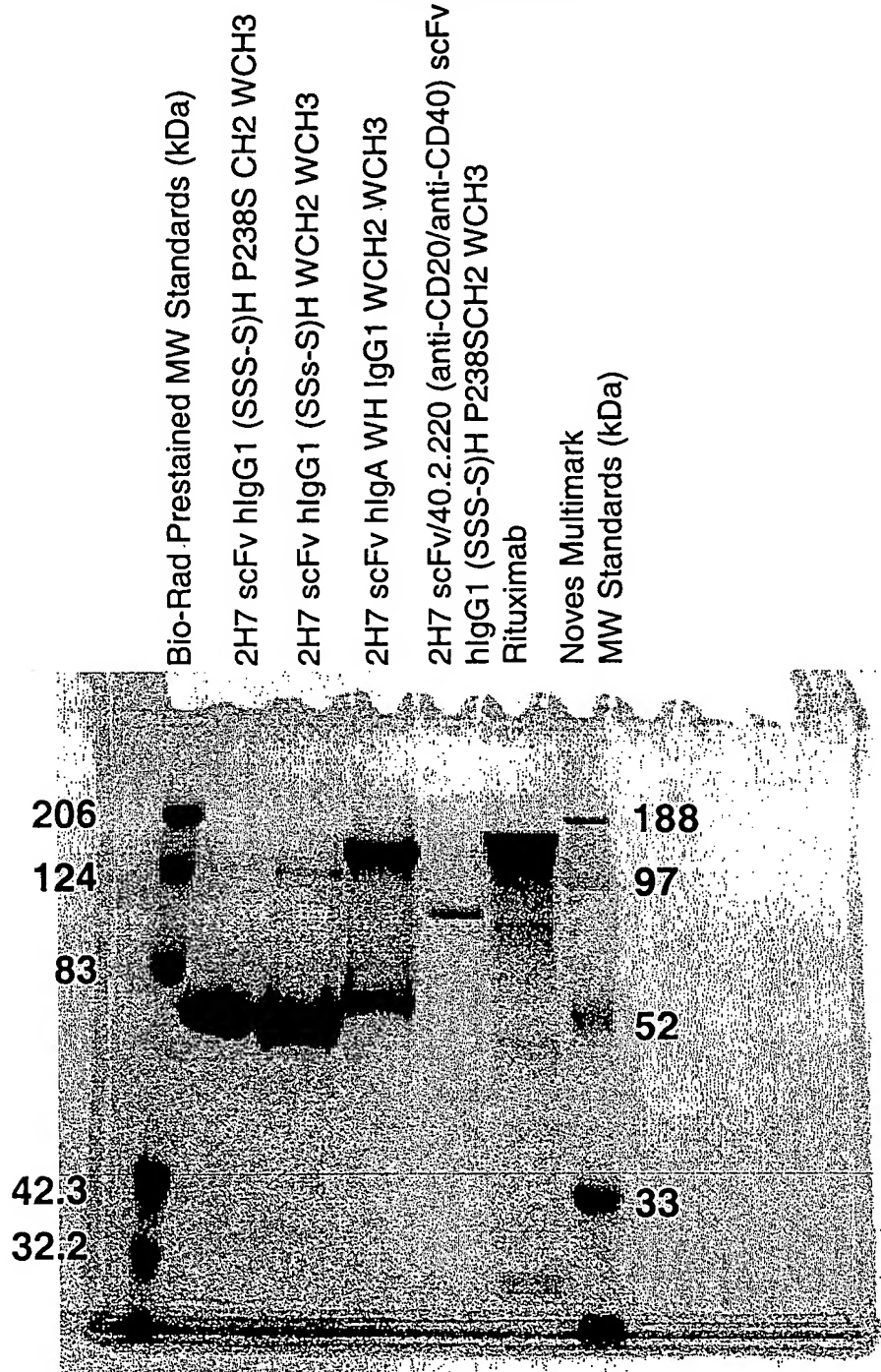
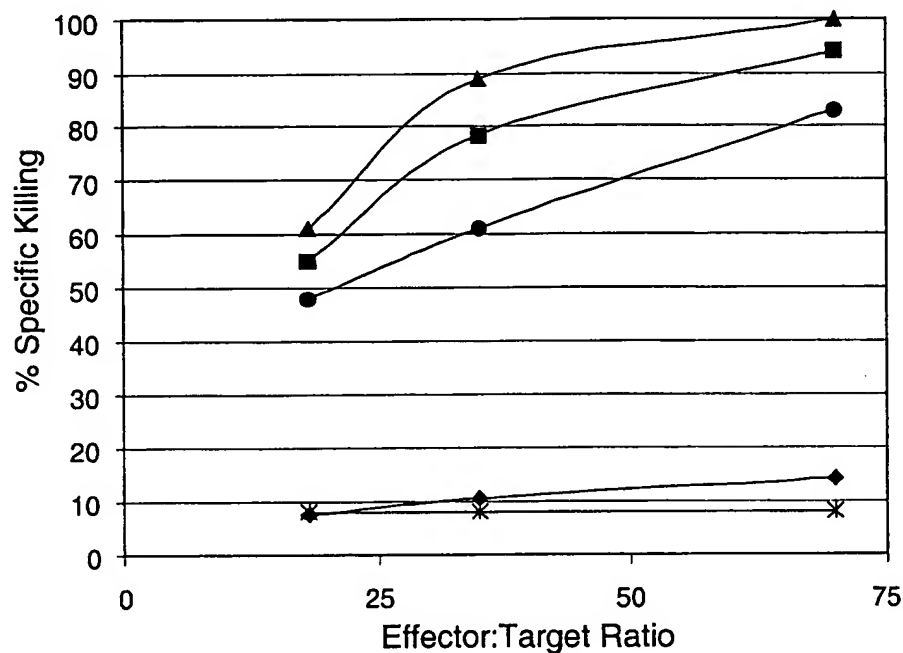


Fig. 46
ADCC mediated by 2H7 scFvIg Constructs by human
PBMC effector cells against Bjab targets

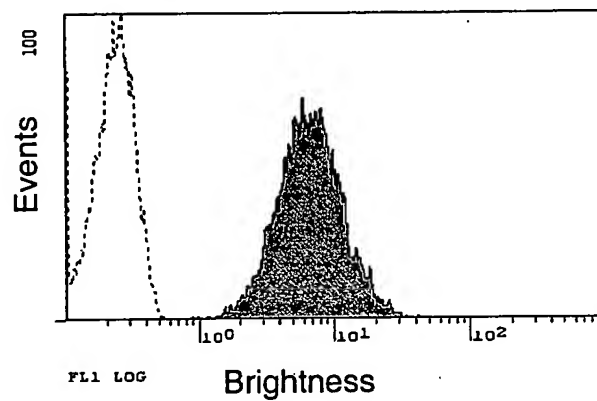


- | | |
|--|------------------------|
| ◆ 2H7 scFv hlgG1(SSS-S)H P238SCH2 WCH3 | ● RITUXIMAB |
| ▲ 2H7 scFv hlgA WH IgG1 WCH2 WCH3 | * CELLS ALONE (W/O AB) |
| ■ 2H7 scFv hlgG1 (SSS-S)H WCH2 WCH3 | |

Fig. 47

Cell surface expression of anti-human CD3 G19-4
scFv hIgG1 (SSS-S)H P238SCH2 WCH3-
hCD80TM/CT on Reh and T51 Cells.

Reh anti-CD3 (G19-4) scFv hIgG1 (SSS-S)H
P238SCH2 WCH3-hCD80TM/CT



T51 G19-4 scFv hIgG1 (SSS-S)H
P238SCH2 WCH3-hCD80TM/CT:

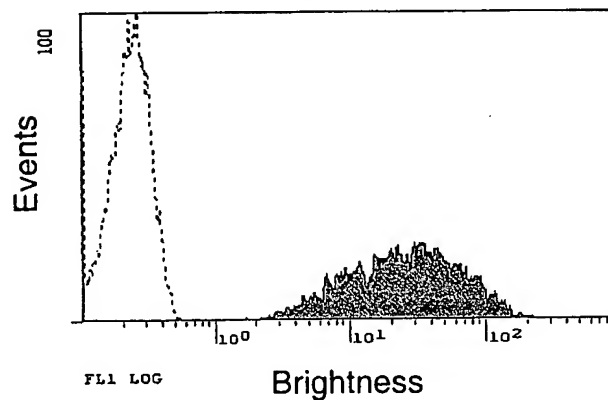
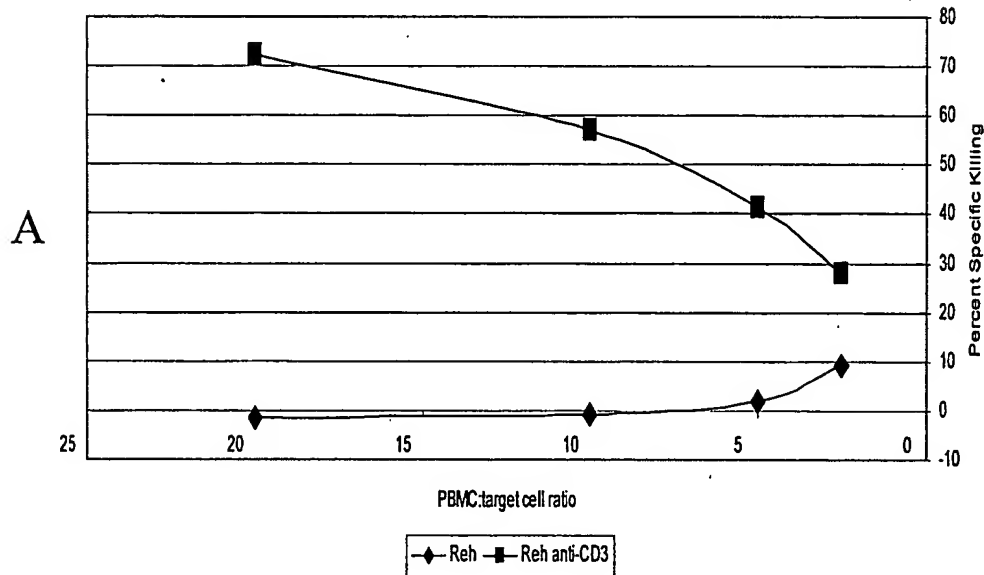


Figure 48.
Targeting of Cytotoxicity to Transfected Cell Lines
by Surface expression of CD3 scFvIg
Cytotoxic activity of resting PBMC towards transfected Reh cells



Cytotoxic activity of resting PBMC towards transfected T51 lymphoblastoid cells

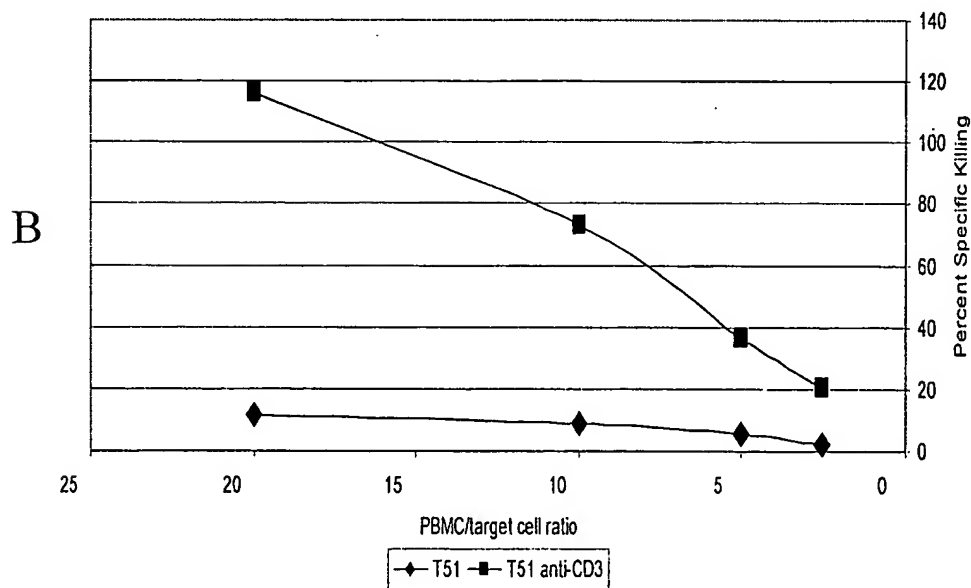


Fig. 49
Binding of 5B9, a mouse anti-human CD137 scFv hIgG1
(SSS-S)H WCH2WCH3 to stimulated human PBMC

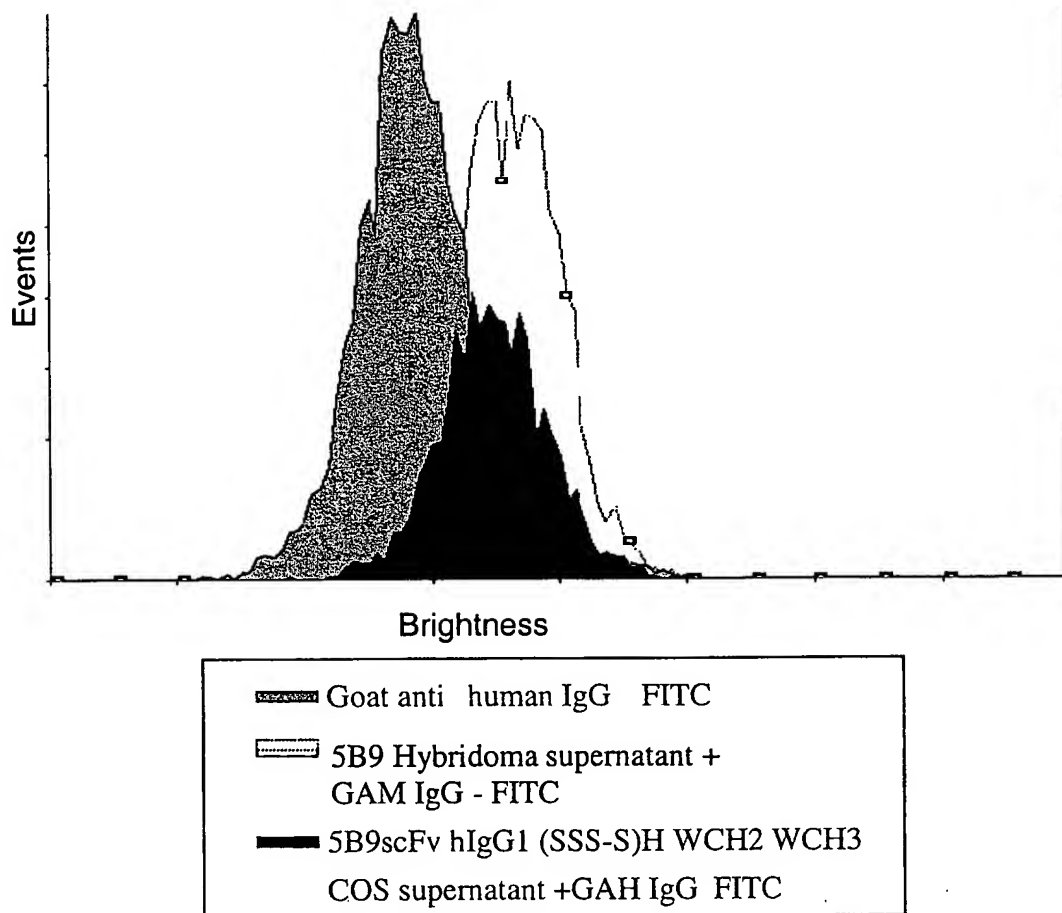
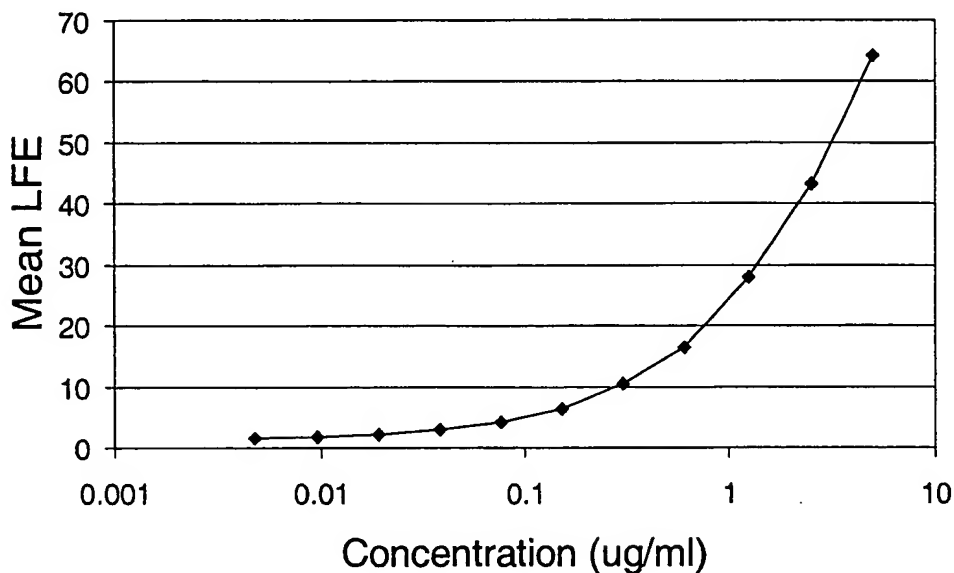


Fig. 50

Effect of V_HL11S Mutation on Cytob20
2H7 scFv hlgG1 (SSS-S)H WCH2 WCH3 Protein Expression

50A. Standard Curve: 2H7VH-L11S-IgG1 (SSS-S)H WCH2 WCH3



50B. CHO supernatant Brightness and Estimation of Protein concentrations from Standard Curve:

	CHO clone name				
	4F2	4F5	3E5	6B11A	2B8A
Mean LFE					
1/100	71.7	40.6	31.5	99.7	101.5
1/500	27.1	12.4	11.2	40.8	43
approx conc. μg/ml	600	225	125	1000	1250

Fig. 51
Production Levels of 2H7scFv VH L11S hIgG1
(SSS-S)H WCH2 WCH3
From CHO Clone Culture Supernatants

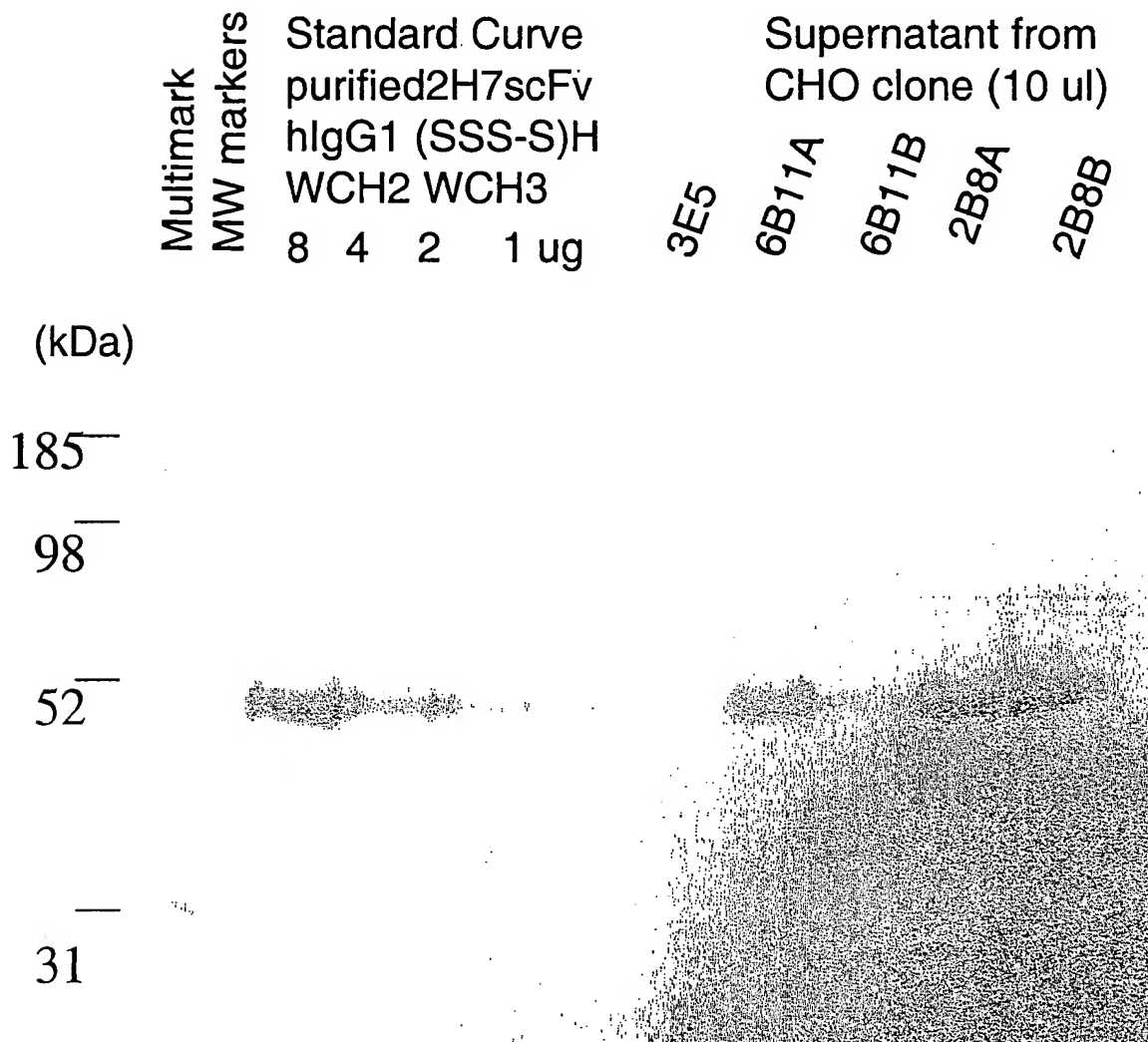


Fig. 52
Effect of VHL11S Mutation on G28-1 scFvIg Construct
Protein Production from COS cells

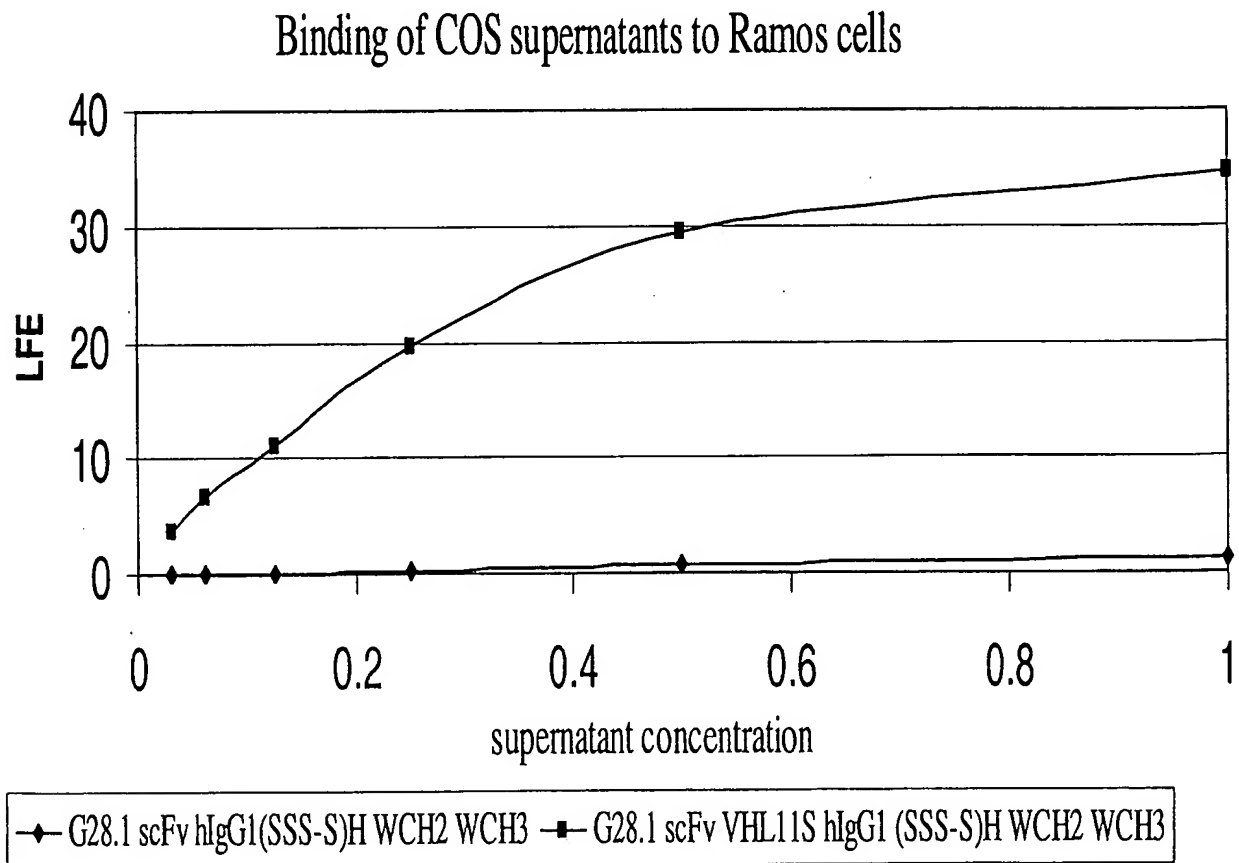


Fig. 53

Immunoblot of G28-1 scFvIg Constructs

Increased Protein Levels in COS supernatants
transfected with G28-1 scFv hlgG1 (SSS-S)H WCH2 WCH3
After Substitution of Leucine with Serine at position 11 of VH (VHL11S)

Fig. 53A.

Purified G28-1 (11/6/01)	G28-1 scFv hlgG1 (SSS-S)H				
scFv IgG1 (SSS-S)H	WCH2 WCH3				
WCH2 WCH3	1 ul/well				
80ng	40ng	20ng	10ng	A	B C D E

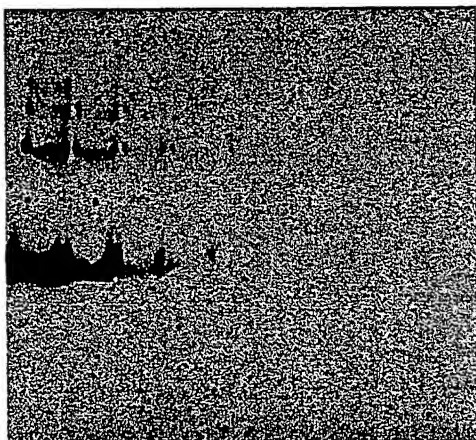


Fig. 53B.

Purified G28-1 (11/6/01)	G28-1VHL11S scFv hlgG1 (SSS-S)H				
scFv hlgG1(SSS-S)H	WCH2 WCH3				
WCH2 WCH3	1 ul/well				
80ng	40ng	20ng	10ng	A	B C D E

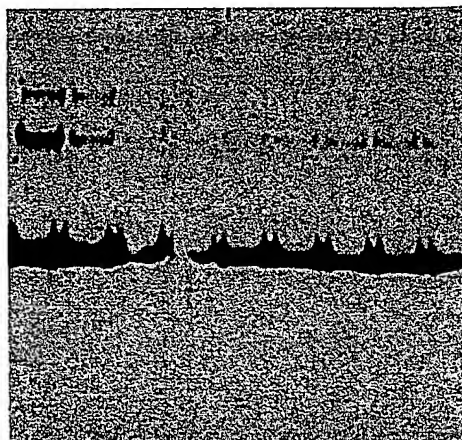


Fig. 54

Binding of 2H7 scFvIg Constructs with Altered
Hinges and CH3 domains to CD20 CHO Cells

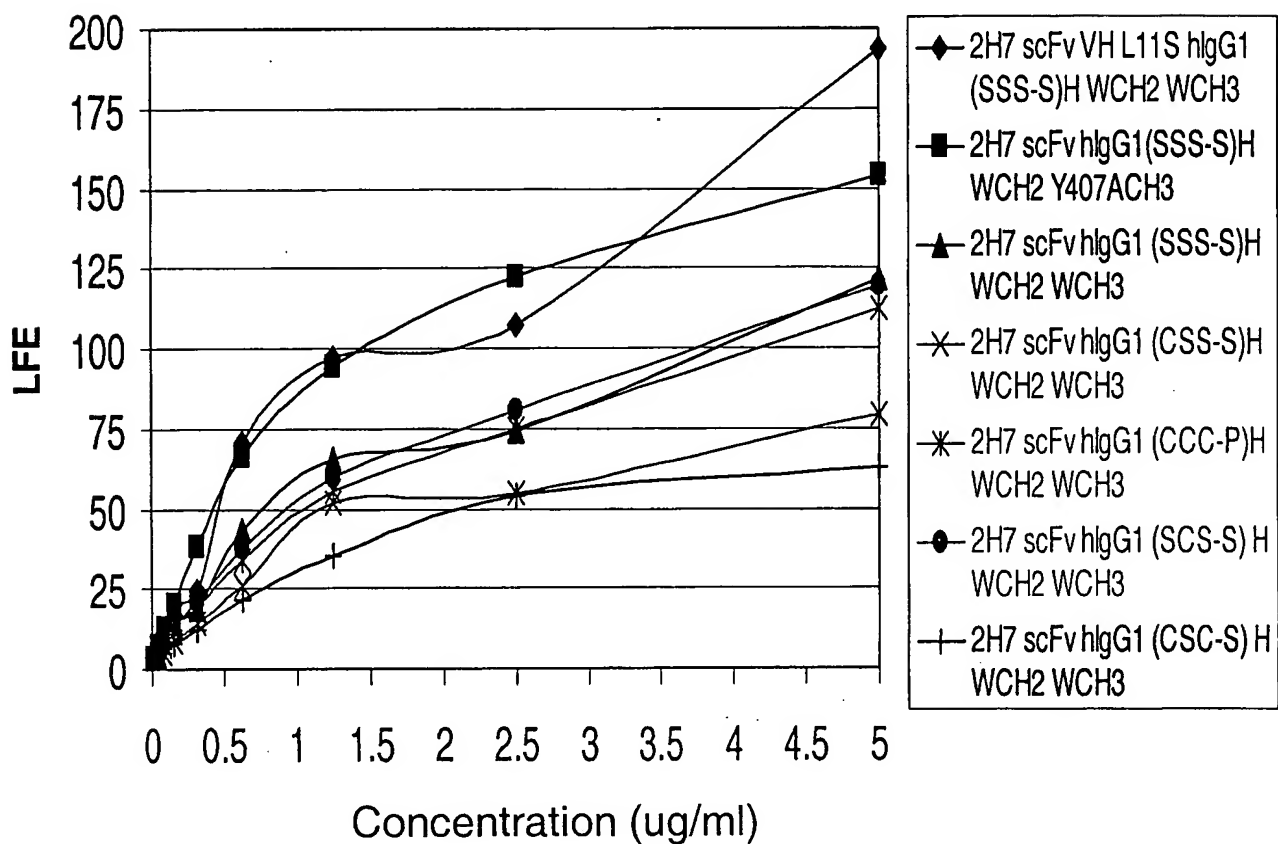


Fig. 55

ADCC Activity of 2H7 scFvlg constructs Against
BJAB Targets and PBMC Effectors

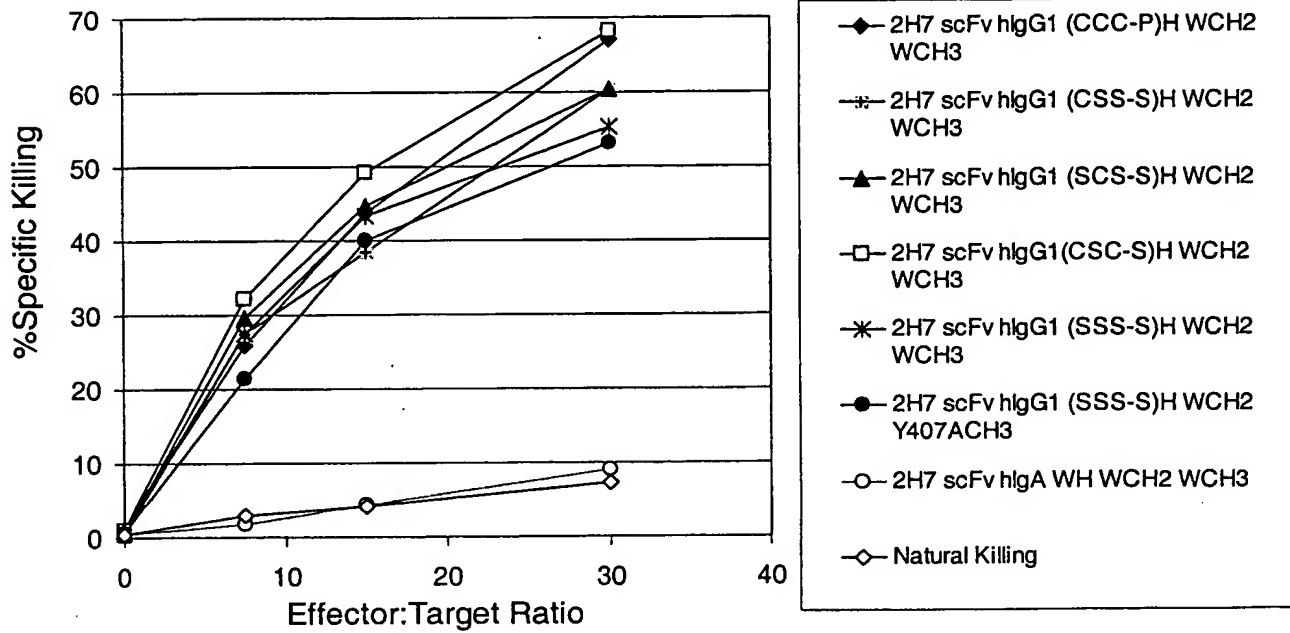


Fig. 56

Complement Activity of 2H7 scFvlg Constructs
With Ramos Target Cells

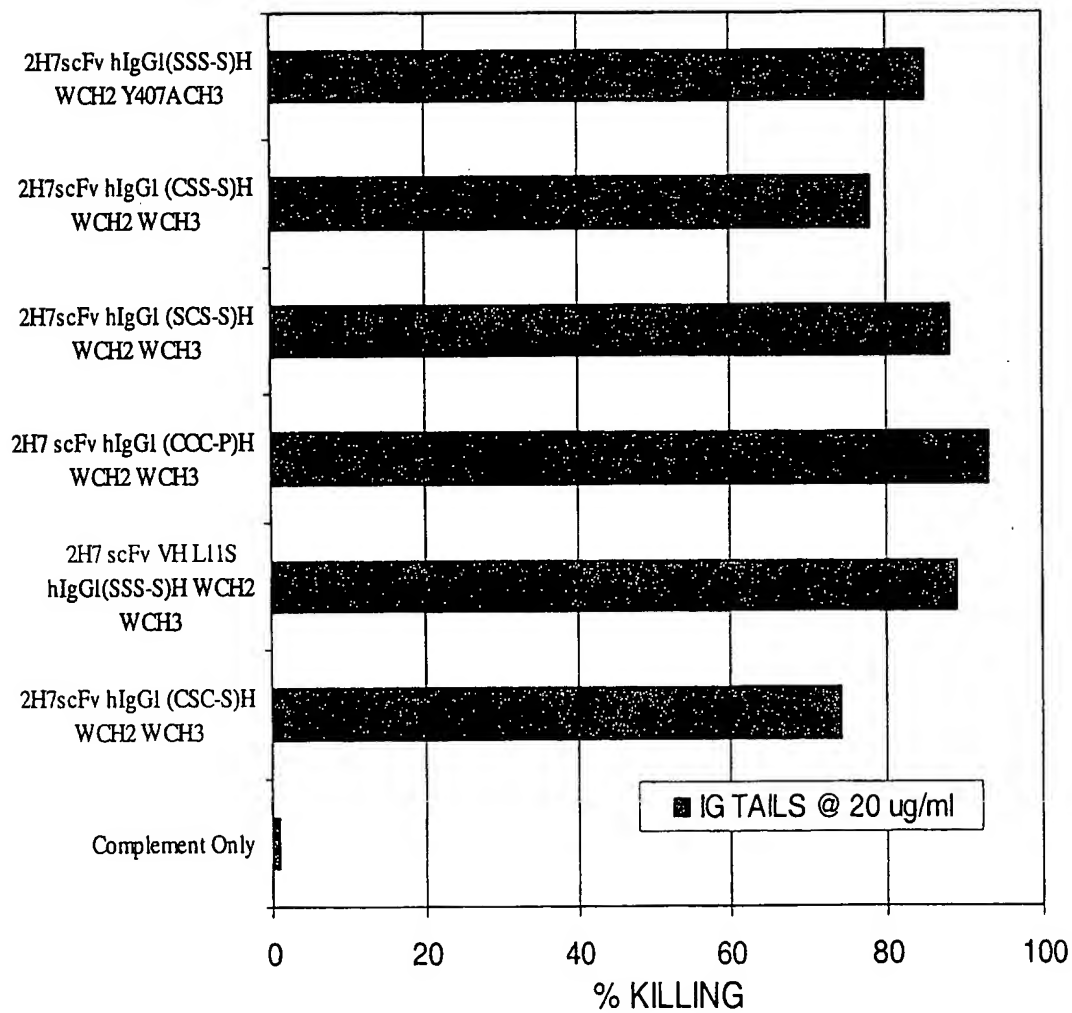
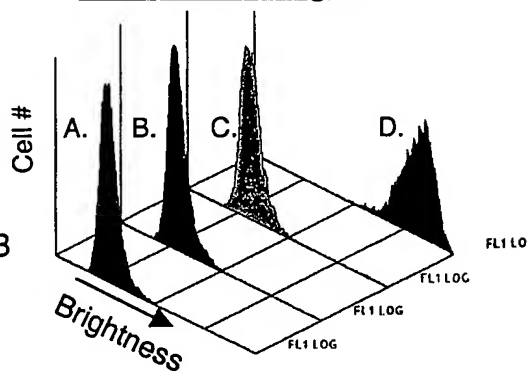


Fig. 57

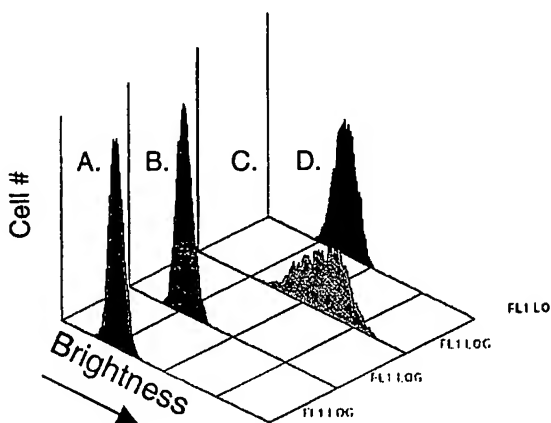
Binding of 2H7 scFvIg Derivatives to CD20CHO Cells

- A. ■ No fusion protein
- B. ■ 2H7 scFv hlgE CH2CH3CH4
- C. ■ 2H7 scFv hlgA WH WCH2 WCH3
- D. ■ 2H7 scFv hlgG1 (SSS-S)H WCH2 WCH3

FITC anti-hlgG



FITC anti-hlgA



FITC anti-hlgE

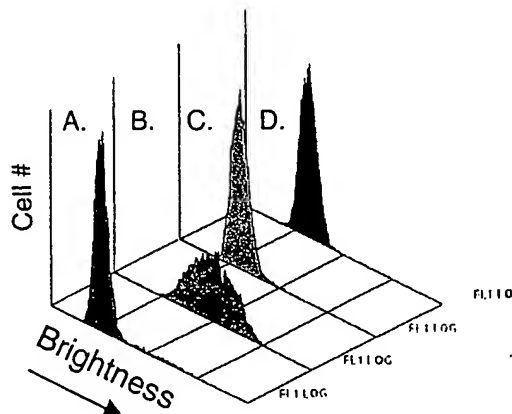


Fig. 58

Fig. 58A. 2H7 scFv VH L11S human IgE (WCH2 WCH3 WCH4)
Binding to CD20 CHO at 30 ug/ml

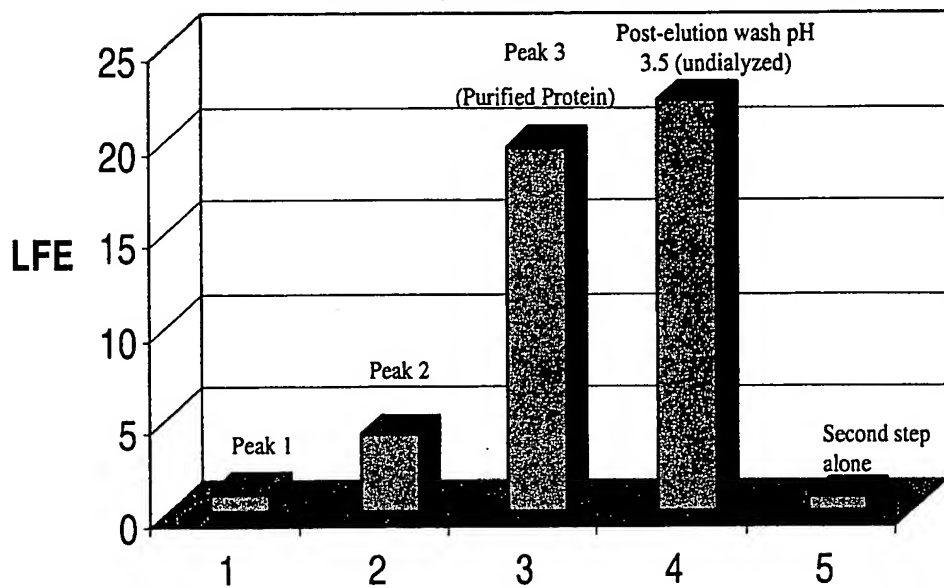


Fig. 58B. ADCC Activity of 2H7 VHL11S IgE (WCH2 WCH3 WCH4)
Protein Fractions with **PBMC** Effectors and Bjab Targets

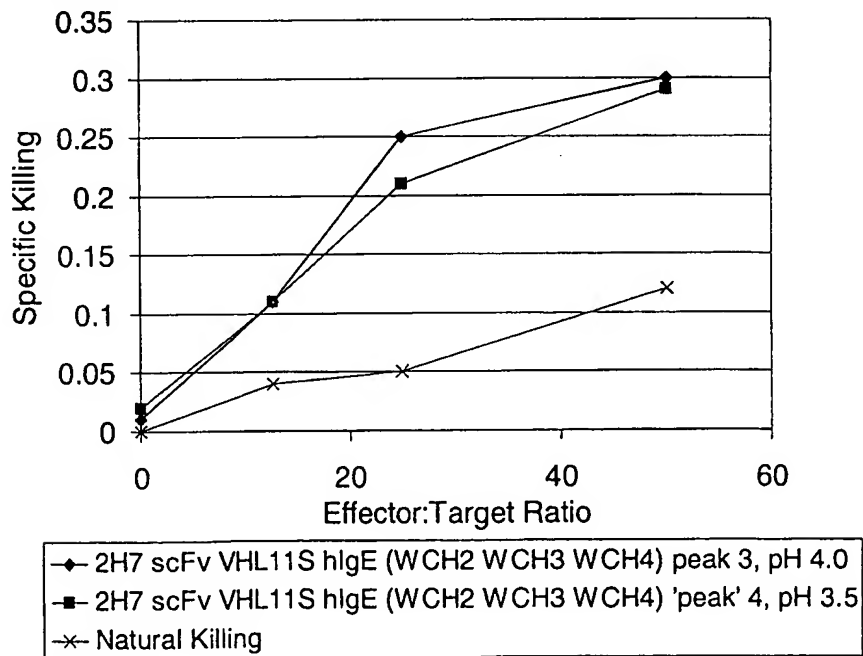


Fig. 59

Binding Data for COS derived α -CD20 (2H7) scFv VHL11S
mIg E (WCH2 WCH3 WCH4) and
mIgA (WH WCH2 WCH3) Tailed Molecules

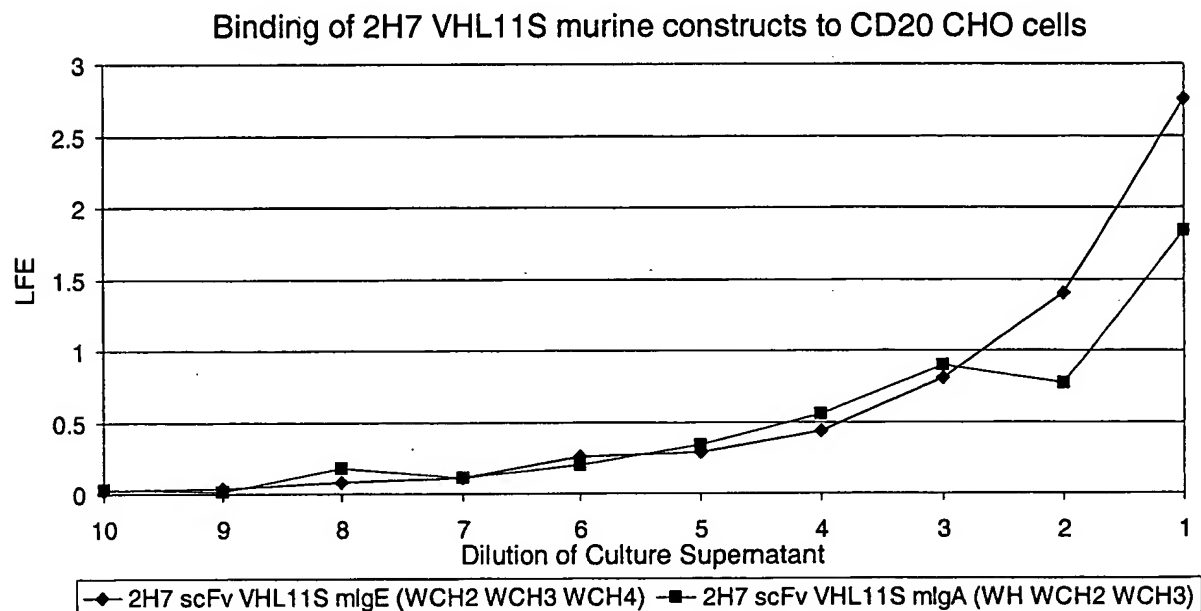
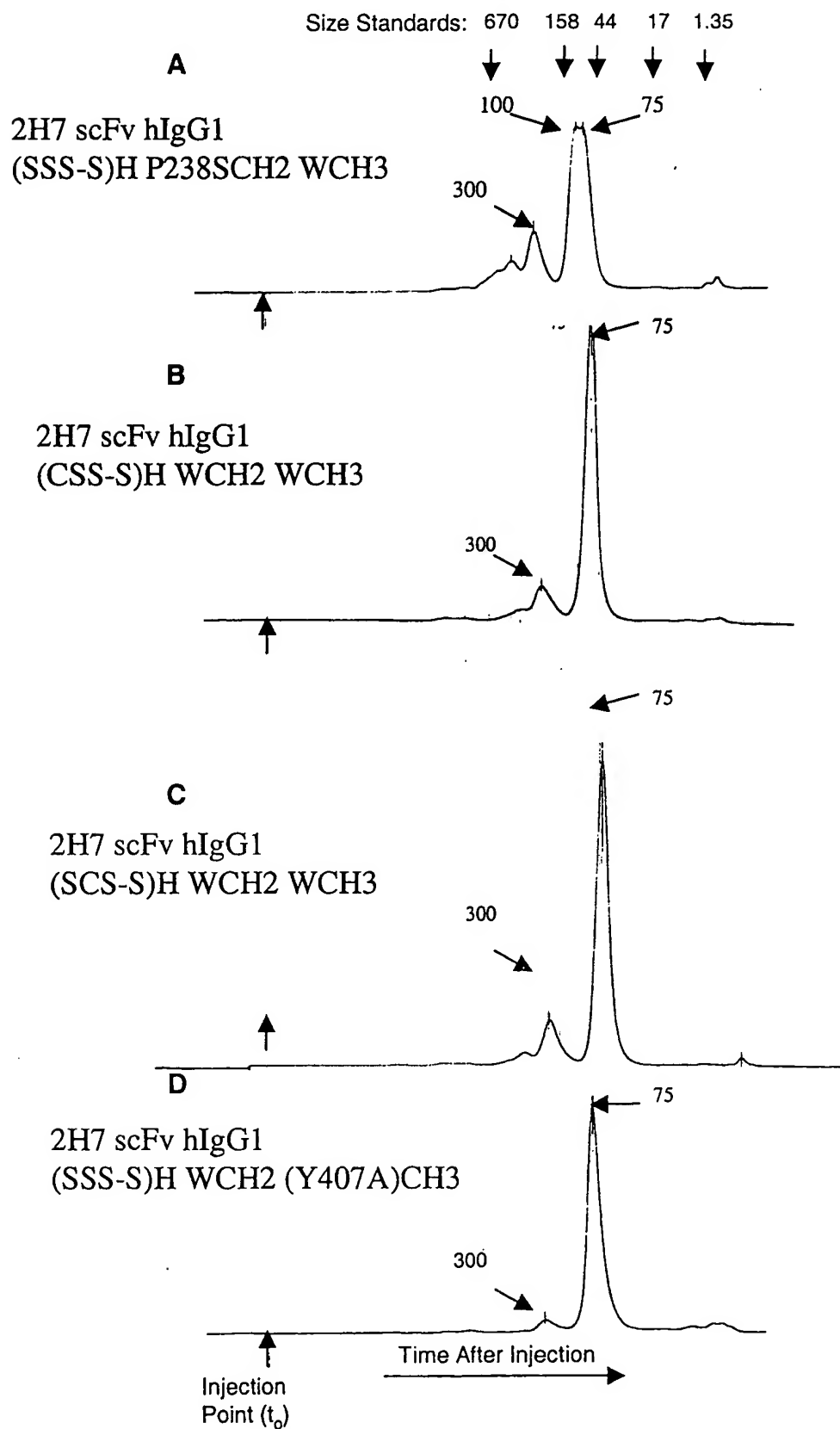


Fig. 60
HPLC Profiles of 2H7 scFvIg Constructs



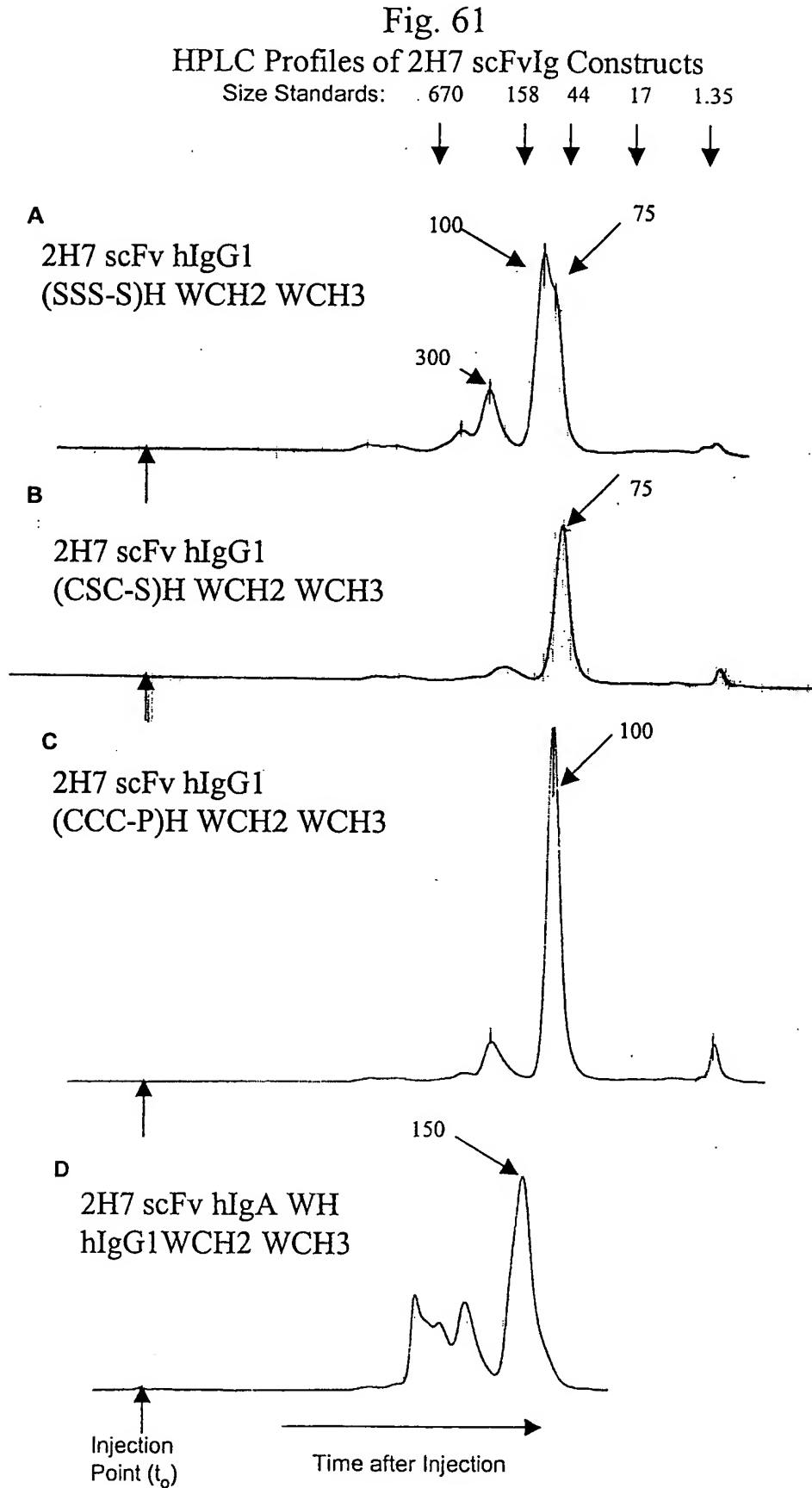


Fig. 62

HPLC Profiles of 2H7 scFvIg Constructs:
Size Standards: 670 158 44 17 1.35

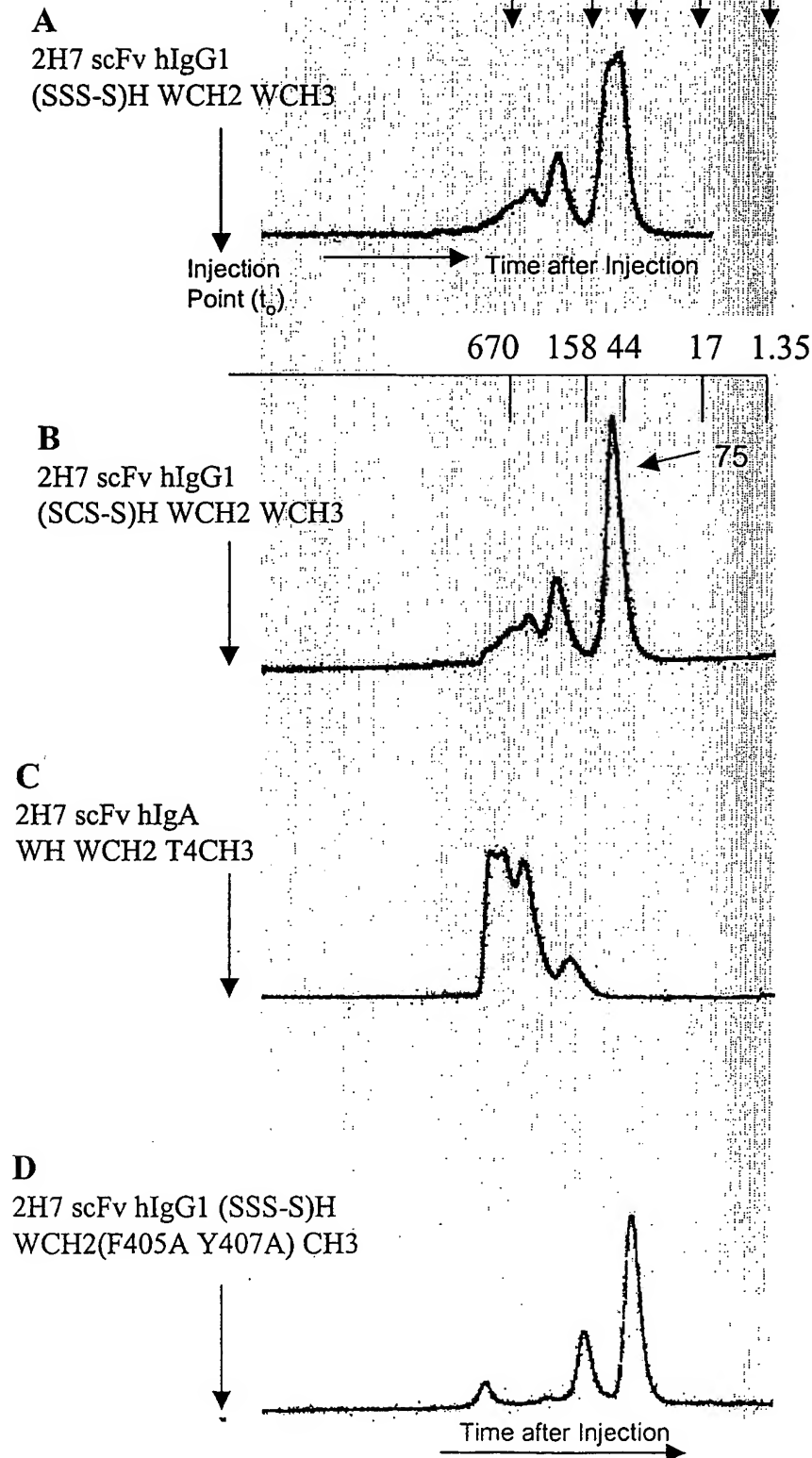


Fig. 63

Binding of Purified Proteins from COS Supernatants
to CD20 CHO cells:
Differential Effects of CH3 Mutations on Binding

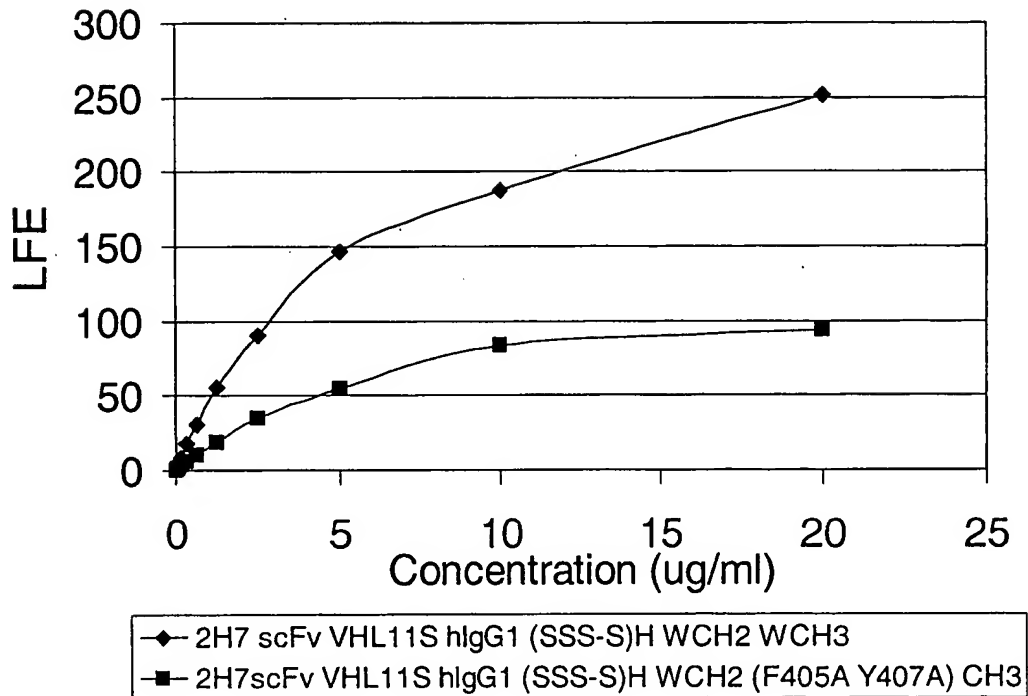


Fig. 64

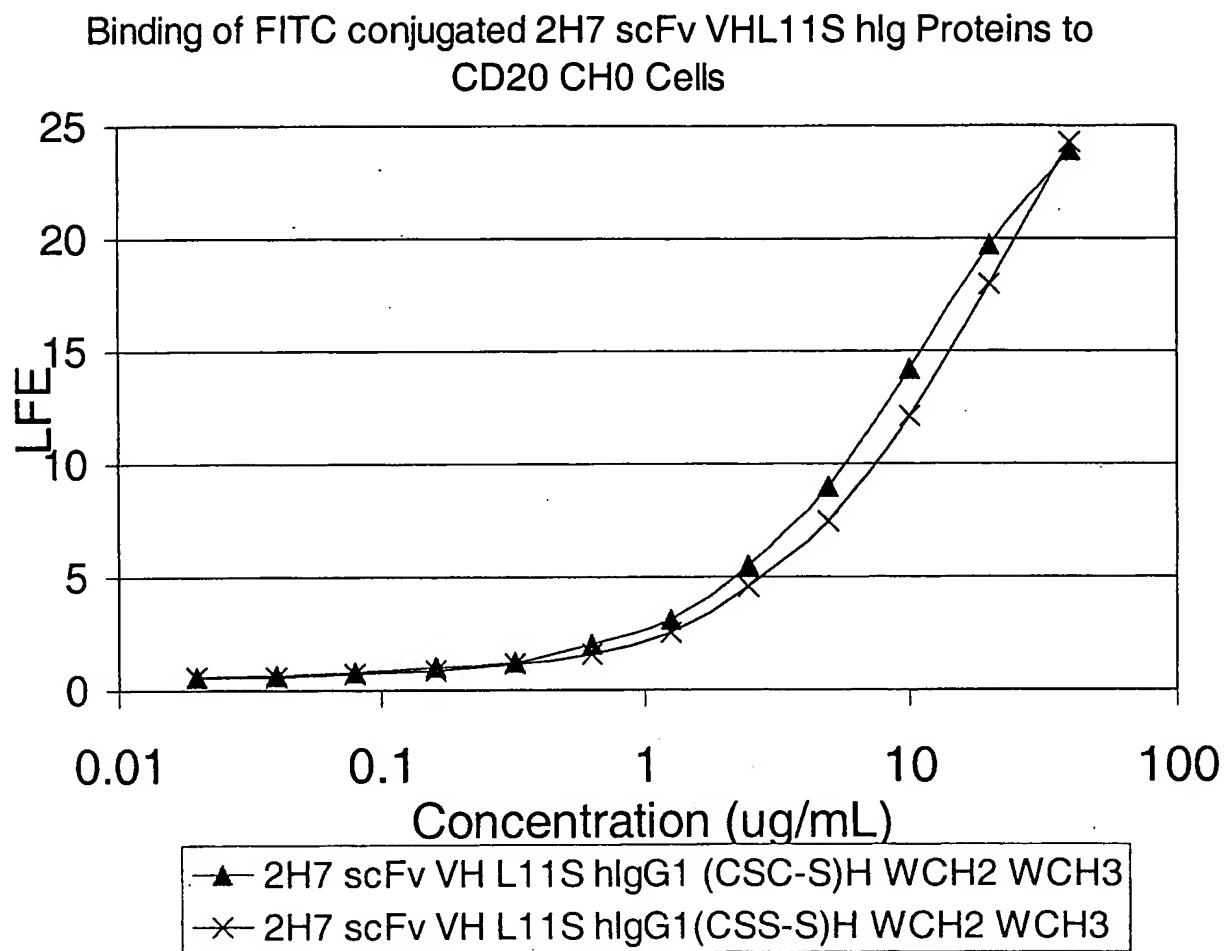


Fig. 65

Nonreducing SDS-PAGE on Protein A-Purified Lots
of 2H7 scFv VHL11S hlg Constructs (10 ug/lane)

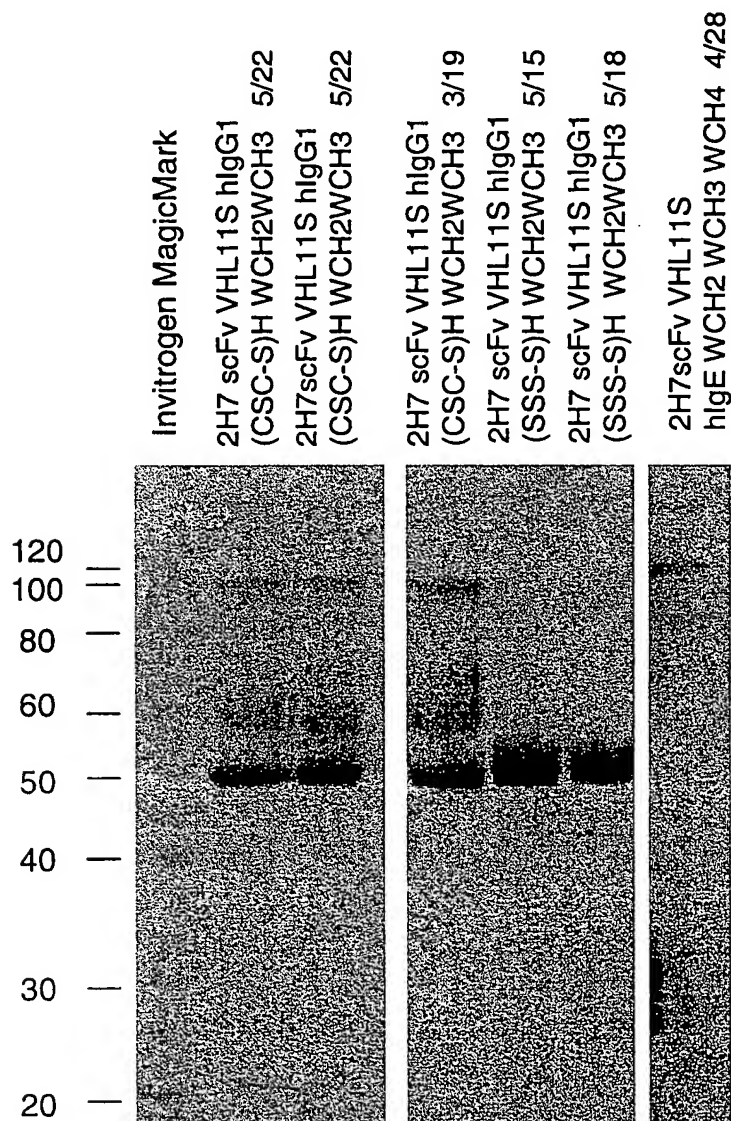


Fig. 66
Alterations in Human IgG Fc sequence
that differentially change effector function efficiency

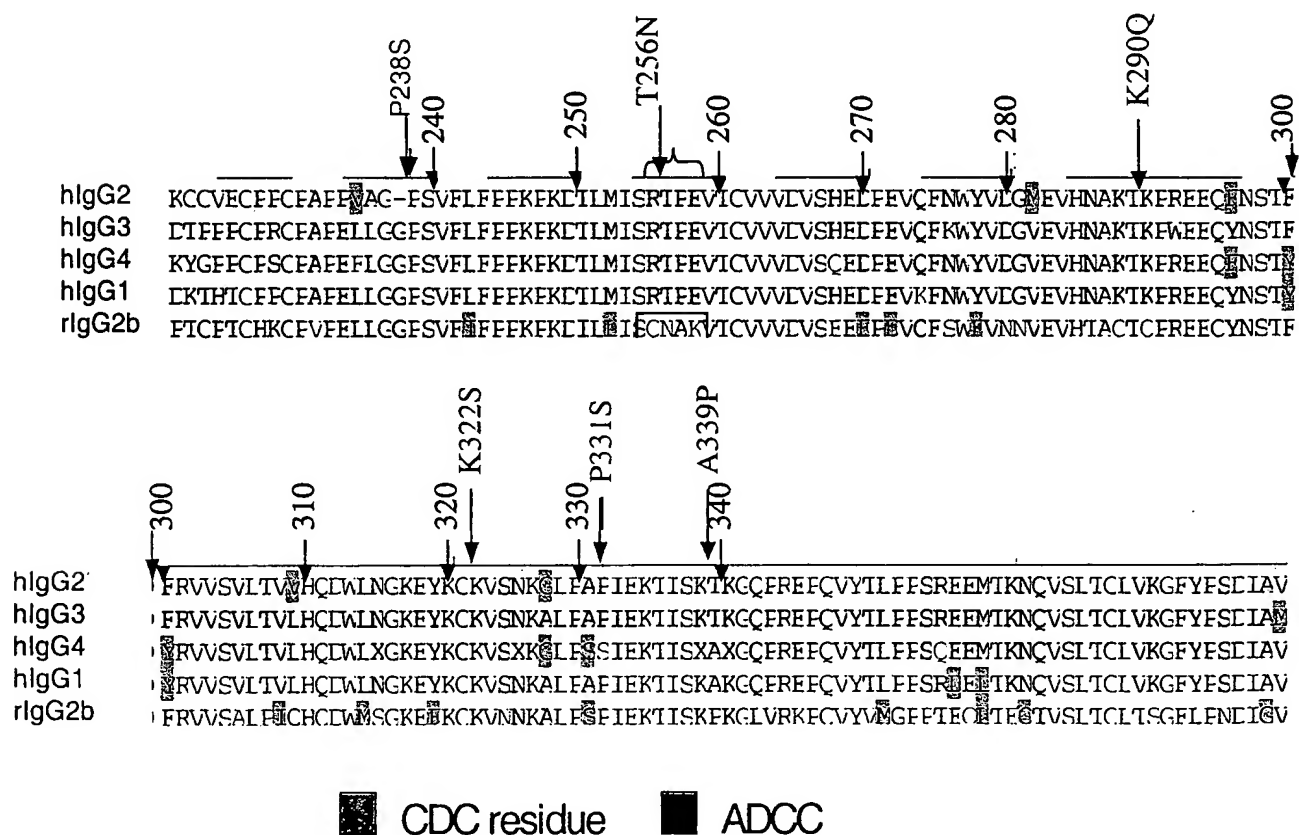


Figure 67.

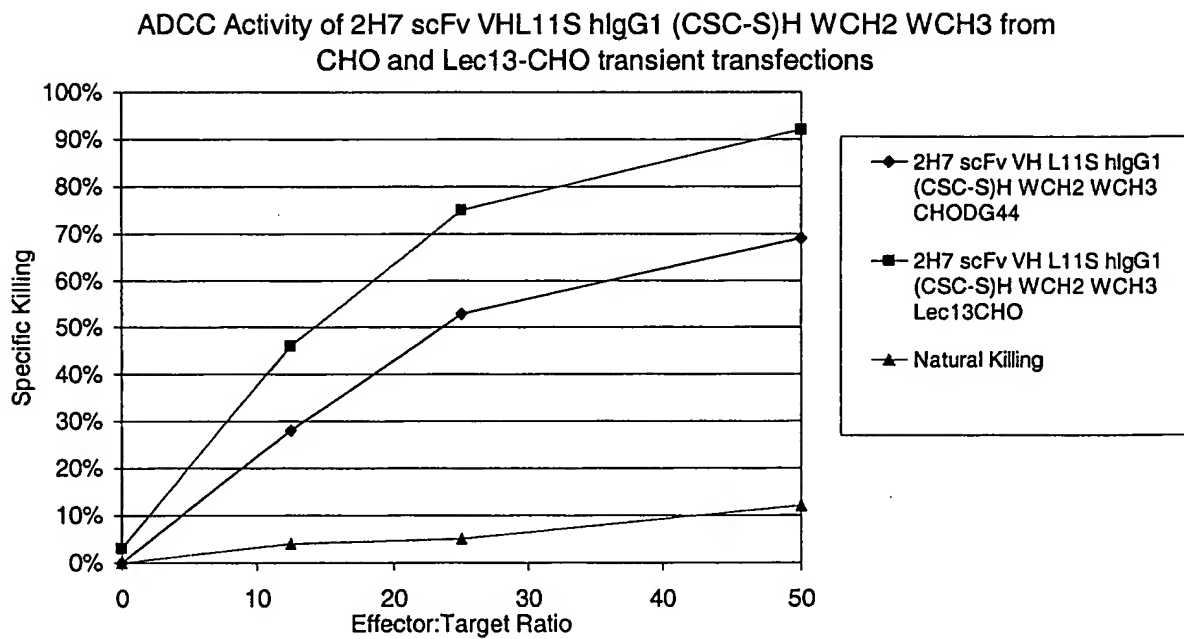


Fig. 68
CD16(ED) hIgG1(SSS-S)H P238S CH2 WCH3 high and
low affinity alleles expressed as soluble molecules

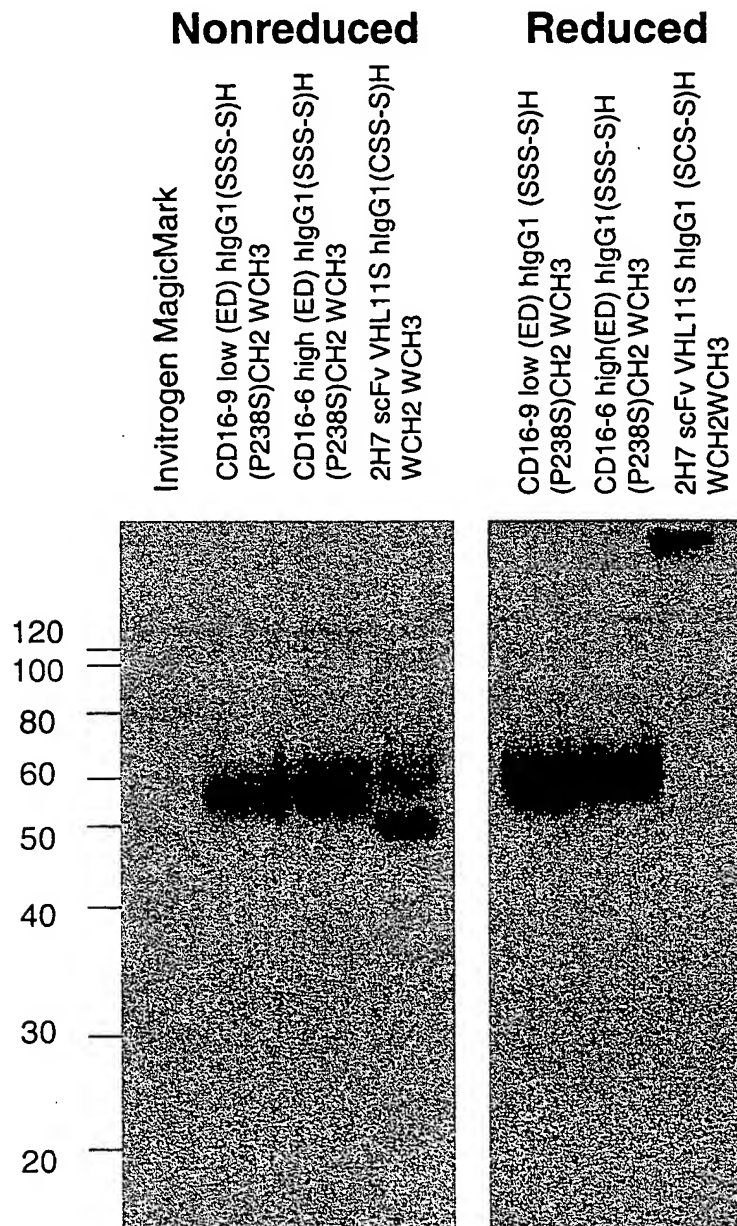


Fig. 69

Binding of soluble CD16-FITC high and low affinity fusion proteins
to 2H7 scFv VHL11S hIgG1 (CSC-S)H WCH2WCH3 or
(SSS-S)H (P238S)CH2WCH3 on CD20CHO Targets

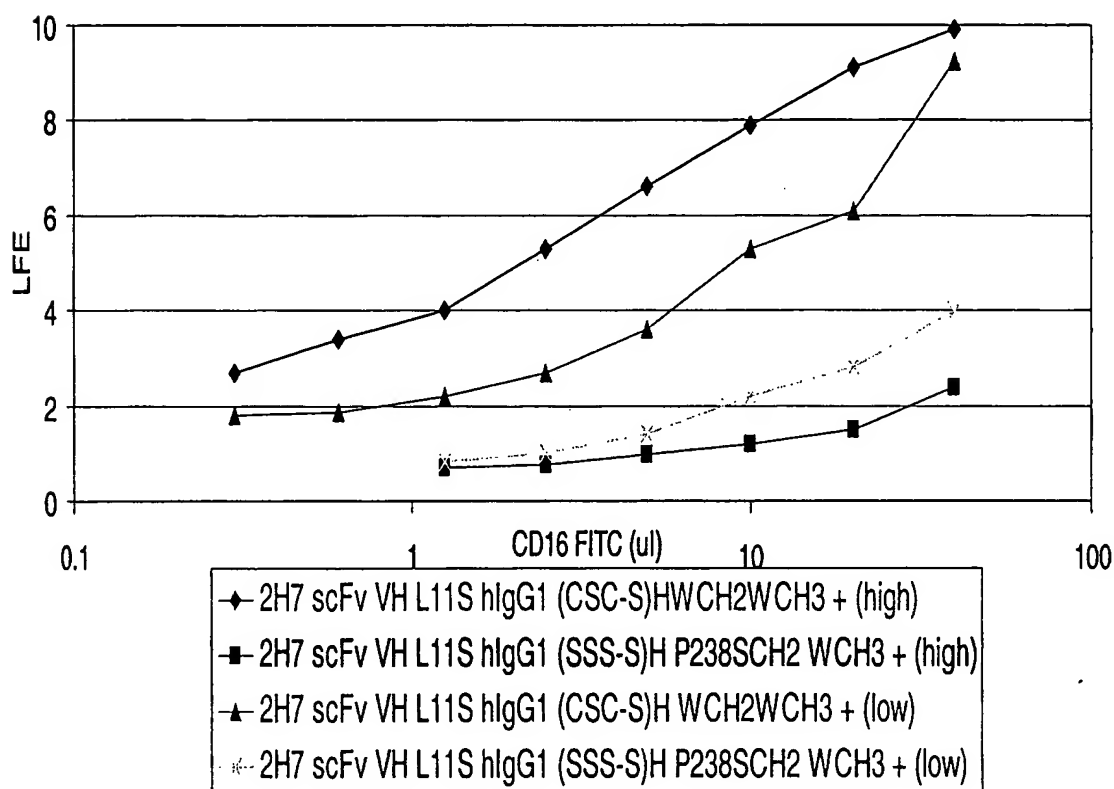
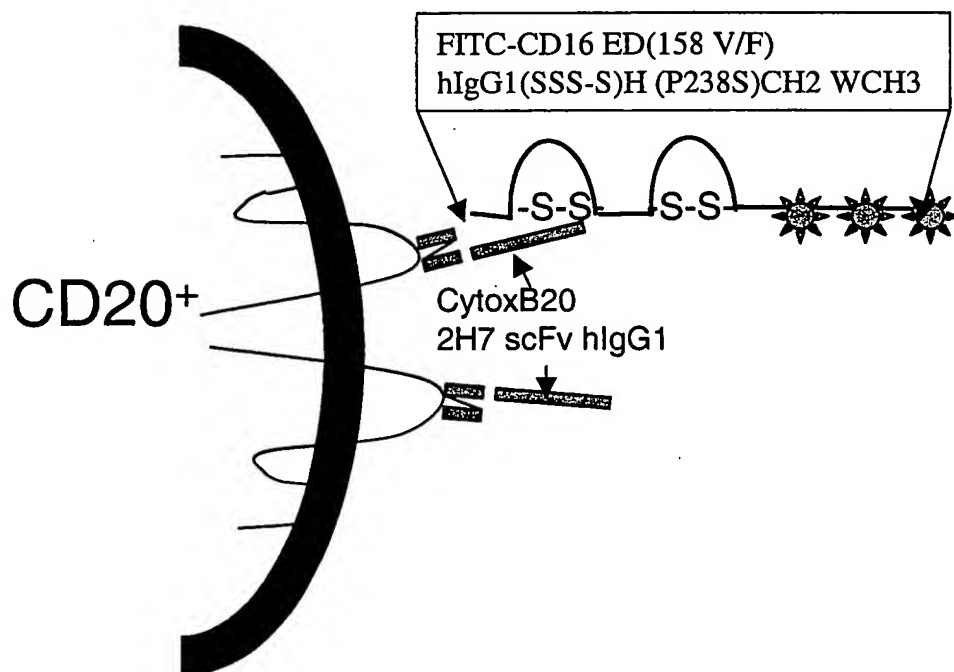


Fig. 70

Binding of FITC Labeled, Recombinant Human
CD16(ED) extracellular domain -Ig Fusion Protein to
CytosB Derivatives on CD20 CHO Cells



Expression of surface displayed SMIPs links
modified cDNAs with the altered fusion proteins

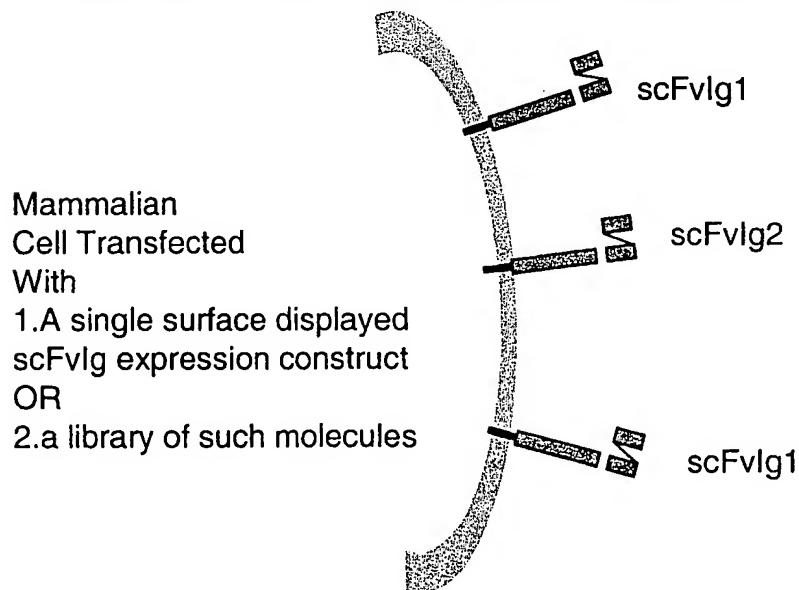


Fig. 71
CD37 mAbs and scFvIg Induce Apoptosis

scFvIg	Bjab Staining	Annexin V Positive	
	No scFvIg	17.5	
	2H7 MH	27	
	G28-1 MH	30.6	
	G28-1 IgAH	28.9	
	HD37 MH	29.1	
	(2H7+G28-1)MH	41	
	(2H7+HD37) MH	37.1	
	(G28-1+HD37) MH	35.3	
mAbs			plus GAM
	Ramos	AnnexinV Positive	AnnexinV positive
	cells alone	3	3.3
	2H7 Mab	1.4	3.1
	G28-1 Mab	18.3	8.7
	HD37 Mab	3.7	3.1
	G28-5	3.9	8.3
	2H7+G28-1	32.3	35.7
	2H7+HD37	5	10.5
	2H7+G28-5	5.7	19.4
	HD37+G28-1	26.9	50
	HD37+G28-5	8.2	18.4
	G28-1+G28-5	39.5	68.3

DECLARATION OF MICHELLE HON

I, Michelle Hon, hereby state and declare as follows:

1. On July 25, 2003, I was employed as a Summer Associate with the law firm of Buchanan Ingersoll LLP, located at 401 West A Street, Suite 1900, San Diego, California.

2. On that date, I assisted James Collett and Barbara Kielt in preparing a U.S. patent application (now assigned Serial No. 10/627,556) for Genecraft, Inc. (now known as Trubion Pharmaceuticals, Inc.).

3. At 10:00 p.m. on that date, while we were trying to print the final version of the patent application in order to take it to the Post Office, the server supporting all of the computers and printers in the office disconnected, and we lost all access to the document and were unable to continue printing. It was not until after sometime after 10:30 p.m. that the server reconnected and we were able to print the patent application.

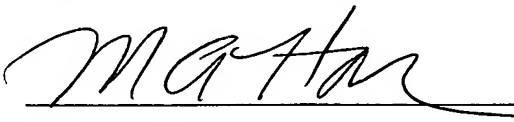
4. The server is located in the main office of Buchanan Ingersoll PC, which is in Pittsburgh, Pennsylvania, and we had no access or control over it.

5. Prior to July 25, 2003, I have received several notifications from the Systems Department in the Pittsburgh office of Buchanan Ingersoll PC that the server would be shut down for maintenance at various times, typically over a weekend. I received no such notification that this would happen on Friday, July 25, 2003.

6. On Monday, July 28, I contacted the Systems Department in the Pittsburgh office of Buchanan Ingersoll PC to find out why the server had shut down. I was informed that it shuts down every night at 1:00 Eastern time in order to create a back-up of the system. I was informed that no notices are sent out for this nightly back-up and I surmise that this is because no one is expected to be affected at this time of night.

I declare that under penalty of perjury that the foregoing is true and correct.

Dated: May 5, 2004


Michelle Hon

DECLARATION OF MILDRED PAMPENA

I, Mildred Pampena, hereby state and declare as follows:

1. On July 25, 2003, I was employed as a Manager of Networks and Communications in the Pittsburgh office of the law firm of Buchanan Ingersoll PC.
2. During July 2003, the Citrix Servers for the entire computer network of Buchanan Ingersoll PC were on a maintenance program under which the servers would automatically shut down every night at approximately 1:00 a.m. Eastern Daylight Time. During this nightly maintenance, the servers take themselves off line for maintenance, which can take up to about 30 minutes or more. During that time, all computer systems that rely on Citrix, including all computer systems and printers in the San Diego office, are unavailable to users.
3. The only way users would know that the computer systems were again available after such an automatic maintenance shut-down would be to try to log on, until they are successfully logged again.

I declare that under penalty of perjury that the foregoing is true and correct.

Dated: June, 2004


Mildred Pampena

DECLARATION OF DALE STUCKEY, JR.

I, Dale Stuckey, Jr., hereby state and declare as follows:

1. I am employed by the U.S. Postal Service at the Midway facility located on Midway Drive, San Diego. I have been employed in this position since approximately 1981.

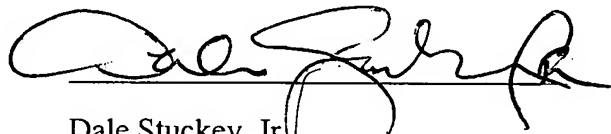
2. One of my responsibilities as a window clerk is to accept incoming mail from customers, including Express Mail packages.

3. On Friday, July 25, 2003 at approximately 11:40 p.m., Barbara Kielt, who I understand to be an Administrative Assistant employed by Buchanan Ingersoll LLP, came to the Post Office with two packages. I was advised that she wanted to send the packages by U.S. Express Mail to the United States Patent Office, and that these packages contained two patent applications.

4. While Ms. Kielt brought the packages to the U.S. Post Office at 11:40 p.m. on July 25, 2003, I did not accept them for U.S. Express Mail, or mark the "Time-In" box on the Express Mail label (Label 11-B) because the window closed at 11:00 p.m. As a result, Ms. Kielt was not able to mail her packages and obtain a date stamp indicating that she presented them for mailing to the U.S. Postal Service on Friday, July 25, 2003.

I declare under penalty of perjury that the foregoing is true and correct.

Dated: August 27, 2003


Dale Stuckey, Jr.

DECLARATION OF JOHN NORTON

I, John Norton, hereby state and declare as follows:

1. I am employed by the U.S. Postal Service at the Midway facility located on Midway Drive, San Diego. I have been employed in this position since approximately 08/01 *John*.

2. One of my responsibilities as the Supervisor: Customer Service Operations is to supervise employees at the Midway facility, and the intake of mail from customers, including Express Mail packages. At this time, I supervise approximately 20 employees at the Midway facility.

3. Dale Stuckey, Jr. is employed under my direct supervision at the Midway facility as a window clerk, and has been an employee of the Postal Service since 1981.

4. Mr. Stuckey has advised me that on Friday, July 25, 2003 at approximately 11:40 p.m., Barbara Kielt, an Administrative Assistant employed by Buchanan Ingersoll LLP, came to the Post Office with two packages. Mr. Stuckey was advised by Ms. Kielt that she wanted to send the packages by U.S. Express Mail to the United States Patent Office, and that these packages contained two patent applications.

5. Mr. Stuckey further advised me that while Ms. Kielt brought the packages to the Midway facility at 11:40 p.m. on July 25, 2003, he did not accept them for U.S. Express Mail, or mark the "Time-In" box on the Express Mail label (Label 11-B) because the window had closed at 11:00 p.m. As a result, Ms. Kielt was not able to mail her packages and obtain a date stamp indicating that she presented them for mailing to the U.S. Postal Service on Friday, July 25, 2003.

I declare under penalty of perjury that the foregoing is true and correct.

Dated: August 27, 2003


John Norton